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VOL. XII ST. LOUIS, MO., DECEMBER, 1926 NO. 3

CLINICAL AND EXPERIMENTAL

AN ANALYSIS OF 500 TUMORS OF THE OVARY*

BY G. L. ROHDENBURG, M.D., NEW YORK CITY

THE material considered in this paper consists of 500 ovarian tumors encountered in an examination of 2691 specimens, in which both ovaries and both fallopian tubes were submitted for diagnosis. On the basis of the present material the tumor incidence may be placed at 18 per cent. The etiology of ovarian tumors, like tumors in general, still remains obscure, and it is still as true as when stated by Frankl in 1914, that a genetic classification of ovarian tumors, which is the only classification of any value, cannot be given with the present state of our knowledge.

Tumors of this organ may be broadly classified on their gross appearance, as primarily cystic with areas of solid tumor, or primarily solid with areas of cystic degeneration, and both of these groups may again be subdivided as regards their innocence or malignancy. One may also group them according to the probable source of origin, as derived from the epithelial structures of the organ, i.e., the follicular or germinal epithelium, or as derived from the connective tissue or endothelium. In the present series 75.9 per cent were primarily cystic, 49.5 per cent being classified as simple retention, follicle, or corpus luteum cysts, 14.2 per cent as cystadenomas, and 12.2 per cent as dermoid cysts and teratomas.

The retention cysts, which are not true ovarian tumors, can be divided into the group of follicle cysts with or without lutein-cell formation, and true corpus luteum cysts. The microcystic ovary, which is not considered as a tumor and is therefore not included in this summary, is interpreted by Frankl as a result of chronic congestion of the ovary, although work which has been elsewhere reported suggests another explanation.

*From the Achelis Laboratory and the Gynecologic Division of the Lenox Hill Hospital, New York City.

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The retention cysts have a central cavity of varying size which contains a fluid varying in color from water-clear to chocolate brown. The cyst wall is formed either of compressed ovarian tissue, or of connective tissue which may or may not present varying phases of degeneration. The corpus luteum cysts represent the dilated and cystic corpus luteum and their diagnosis is based upon the demonstration of granulosa luteum cells in the characteristic

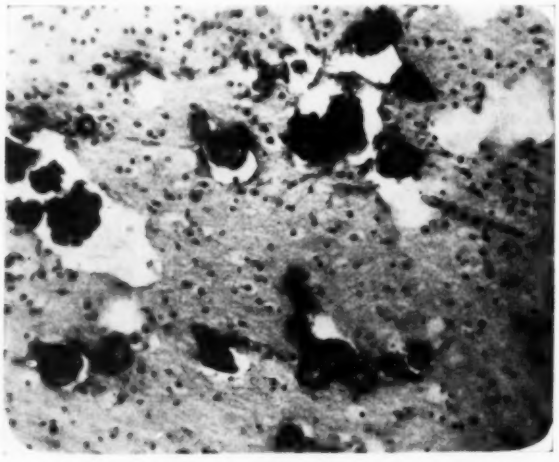


Fig. 1.—Pacchionian bodies in teratoma. x250.

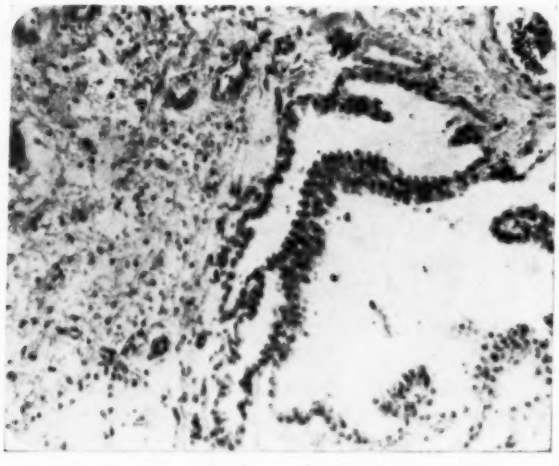


Fig. 2.—Choroid plexus in teratoma. x250.

arrangement of the corpus luteum. This cellular layer is rarely present in the larger cysts.

Our material adds nothing new to the present knowledge of these lesions. In the present series one specimen was noteworthy because of its size, the weight being 42 kilos.

Cystadenomas filled with a mucous stringy fluid are the most common of the ovarian tumors, constituting 53.6 per cent of Lippert's 638 ovarian

tumors, while our material showed 71 cases (14.2 per cent). As a rule only one ovary is affected (both ovaries in less than one-quarter of the cases, according to Frankl). These tumors possess a connective tissue capsule and a uniformly rounded surface, or multiple constrictions according to the dominance in size of different compartments. The size of the individual cysts is variable and in unilocular cystomas one large cyst is seen which may or may not show a variable number of cysts or cystlike spaces in the wall. Some of

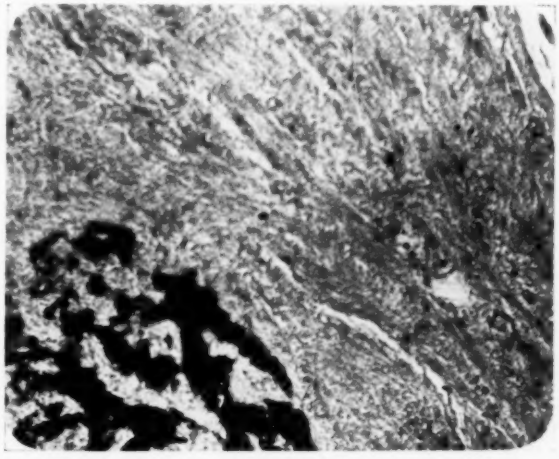


Fig. 3.—Retinal pigment in teratoma. x250.

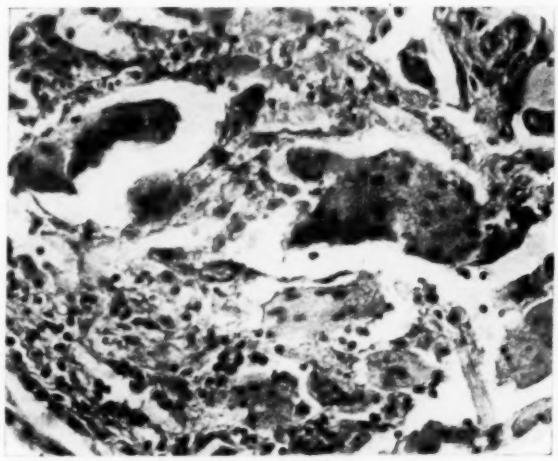


Fig. 4.—Lipoidal reaction in wall of dermoid cyst. x250.

these tumors present an adenomatous structure and closely resemble solid tumors of the ovary. Generally speaking the cystadenomas belong to the group of benign tumors, but in spite of their histologic appearance they may at times assume the properties of a malignant neoplasm. Peritoneal implantations not infrequently manifest themselves after the spontaneous or accidental rupture of a pseudomucinous cystoma. Although it is not a frequent occurrence, these tumors sometimes undergo a histologically demonstrable

carcinomatous degeneration affecting both the primary tumor and peritoneal implantations. Such degeneration is more common in the serous cystadenomas, especially those of the papillary type. The later group contain a serous instead of a mucinous fluid and the inner surface of the cyst spaces is relatively often covered with papillary epithelial growths. Any of the cystadenomas may reach enormous dimensions.

Of greater interest than those previously described is the group of 61 dermoid cysts and teratomas. Kocher claims that the diagnosis of ovarian teratoma or dermoid cysts is rendered possible by the "malleability" of the tumor, meaning by that term that slow prolonged pressure produces a distinct depression which persists for some time. This is due to the putty-like consisteney of the cyst contents. Where hair, fluid, and fatty masses are present it is claimed that a fine crepitation may at times also be demonstrated.

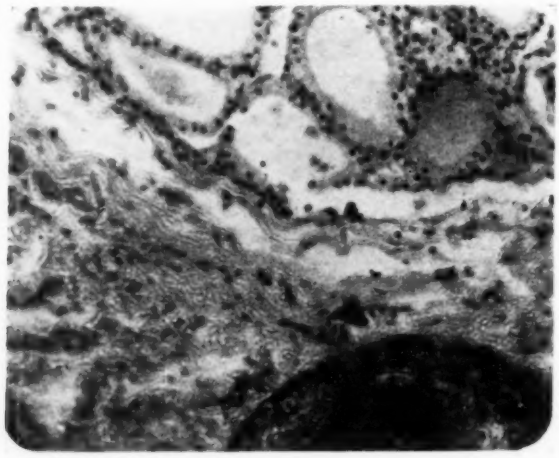


Fig. 5—Thyroid tissue in teratoma. x250.

In the present series several points which have been seldom described were encountered. Eighteen of the group were pure dermoid cysts in which the only tissue type demonstrable was the skin and its appendages. In 22 others in which the skin and its appendages formed the major portion of the growth all three fetal layers were demonstrable upon careful study.

In the pure dermoid cyst group, the histologic picture is that of a central cavity filled with sebaceous material and hair, and surrounded by a wall the inner surface of which is a more or less keratinized skin with the usual appendages of sebaceous glands, hair follicles, etc. This epithelial layer is in turn supported either by a connective tissue layer, or by a condensation of the ovarian structure. Although seldom commented upon, it was not surprising that 9 instances of lipoidal reaction were encountered. The large amount of lipoid present in the sebaceous material is the explanation, and the reaction, which is shown in Fig. 4, is characterized by the formation of foreign body giant cells.

The frequency with which certain tissues were found in teratomas furnished another interesting observation. Brain was encountered 34 times.

and once the frequency of pacchionian bodies produced a picture analogous to a psammoma (Fig. 1). In one specimen a fully developed choroidal plexus was encountered (Fig. 2). Retinal pigment with partial development of the retina was found in 6 instances (Fig. 3). Thyroid tissue was demonstrable in 9 specimens (Fig. 5), while cartilage, bone, or teeth were found in all of the group.

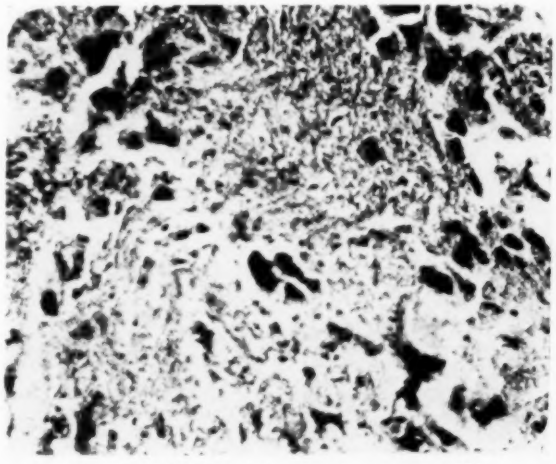


Fig. 6.—Chorioepithelioma of ovary. x250.

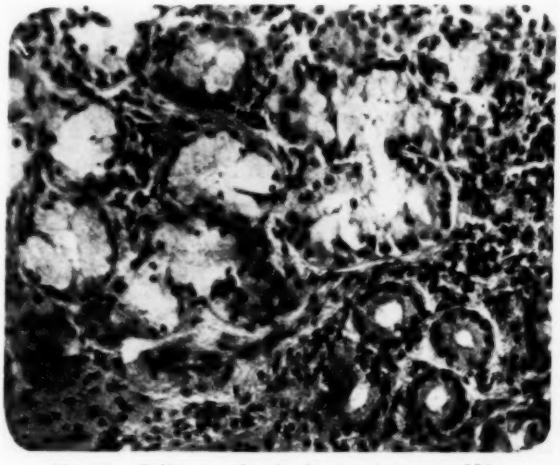


Fig. 7.—Salivary glands in teratoma. x250.

Malignant degeneration of the teratomas occurred in 6 specimens (10 per cent). Four of these 6 were classed as carcinomas, the cells being of epithelial origin. In all, alveolar formation was a prominent feature and special stains showed no intercellular fibrils of connective tissue. In one of the malignant tumors (Fig. 11) a squamous-celled epithelioma developed in an area of skin, and metastatic or direct growth extension was demonstrable in the bone and cartilage of the tumor itself.

One instance of carcinosarcoma was shown to be of teratomatous origin by the presence of islands of bone, skin, and muscle.

Solid tumors of the ovary are possibly of greater interest than the cystic ones, because they are less frequently encountered. The least common is the adenofibroma, 0.8 per cent of the present material being thus classified. Of the four tumors in this group, three belong to one type and the fourth to another. In the first (Fig. 18), the body of the tumor consists of a hyaline or

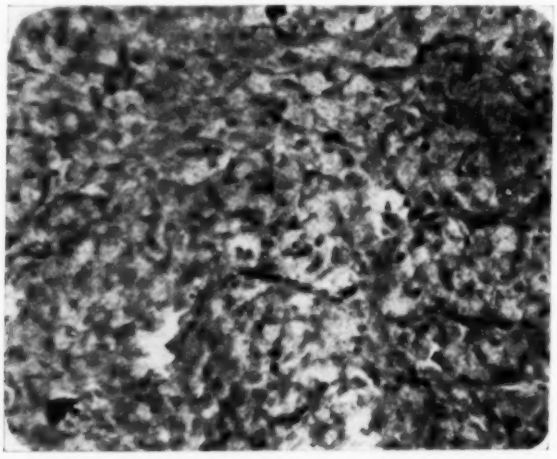


Fig. 8.—Krunkenberg tumor (primary in ovary). x250.

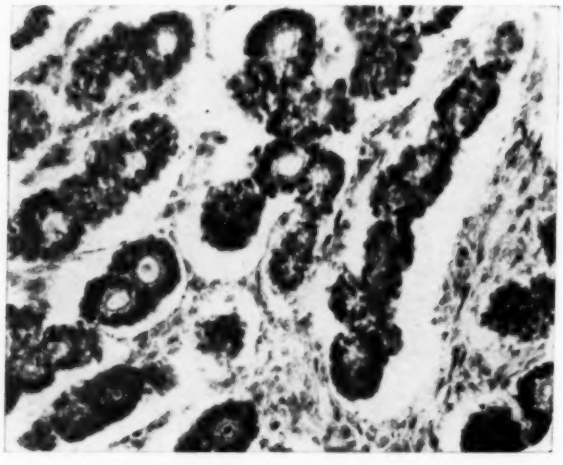


Fig. 9.—Folliculoma ovarii. x250.

slightly cellular stroma arranged with the peculiar manner of infolding observed in intracanicular tumors of the breast. These papillary-like ingrowths of connective tissue were lined by a single layer of low columnar or cuboidal epithelium which gave no evidence of secretory activity. The single tumor had a more cellular stroma which supported alveoli of varying size, generally ovoid in shape. Most of the cells of these alveoli (Fig. 17) showed evidences of secretory activity, and cilia were occasionally demonstrable. Many of

the secretory products had become calcified. This last type is possibly derived from embryonal rests which are not infrequently encountered in the ovary. We have several times observed proliferations of connective tissue about misplaced embryonal ducts, of such degree and so sharply localized, that the diagnosis of fibroadenoma of microscopic size was justifiable.

Sixteen specimens of papilloma (3.2 per cent) were found in the tumor group, and as with fibroadenomas there were two types distinguishable. In

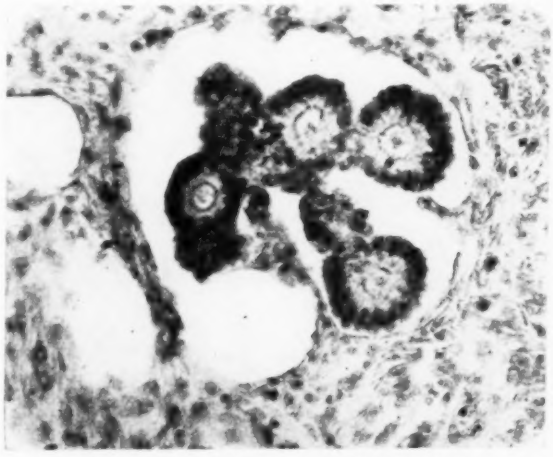


Fig. 10.—Folliculoma ovarii. x350.

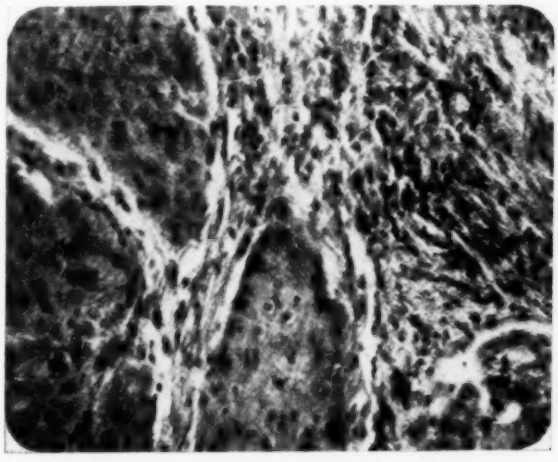


Fig. 11.—Squamous-cell epithelioma arising in teratoma. x250.

the first type (Fig. 12) the architecture consisted of a dense and rather hyaline connective tissue arranged as papillae and covered by a single layer of low columnar epithelium without evidence of secretory activity. Of this type there were 8 examples. The second type (Fig. 13) had in general a looser connective tissue stroma also arranged in papillary fashion and covered by an epithelial layer which varied from low to high columnar. The characteristic difference between the two types was that the epithelial covering of the

second was of a secreting type, while the epithelium of the first type was nonsecreting.

Fibromas of the ovary are solid tumors which usually have a nodular surface. They vary in size from the diameter of a pea up to 40 kilos in weight. The general form of the ovary may have been preserved or it may have merged into the tumor, and usually the tumors are pedunculated. Bilateral tumors are seldom observed. At times the histologic differentiation

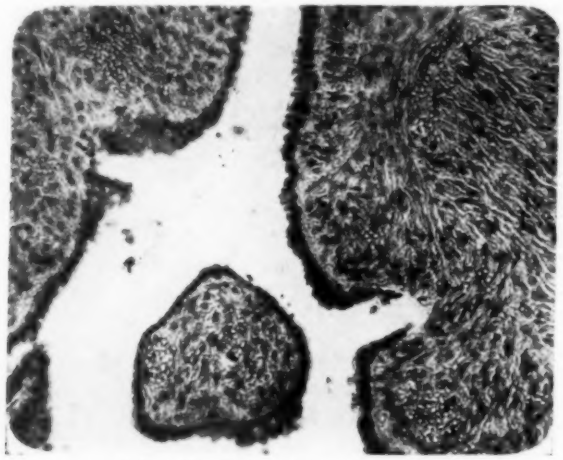


Fig. 12.—Fibroadenoma. x250.

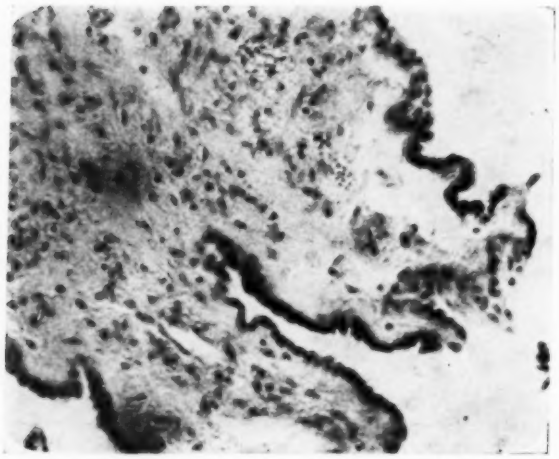


Fig. 13.—Papillary cystadenoma (nonsecreting type). x250.

from sarcoma is difficult, and occasionally the tumors contain varying amounts of smooth muscle. While fibromas are currently supposed to be rather infrequent in the ovary, there were 23 in the present series (4.6 per cent). In general the tumors consisted of interlacing fasciculi of connective tissue which varied markedly in the degree of cellularity, in many instances being hyaline and in other instances so cellular as to arouse a suspicion of malig-

nancy. In some the vascular supply was particularly abundant, but in the majority the blood vessels showed a marked degree of sclerosis.

Two possible sources of origin were demonstrable in the series. In one specimen (Fig. 19) in which there were multiple fibromas, small tumors could be definitely shown to arise from the adventitia of small blood vessels. A similar source of origin has also been shown for fibromas in other portions of the body. In another specimen (Fig. 20) an area of struma ovarii in the

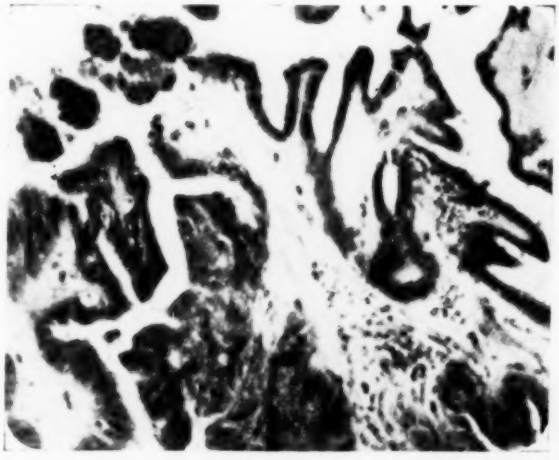


Fig. 14.—Papillary cystadenoma (secreting type). x250.

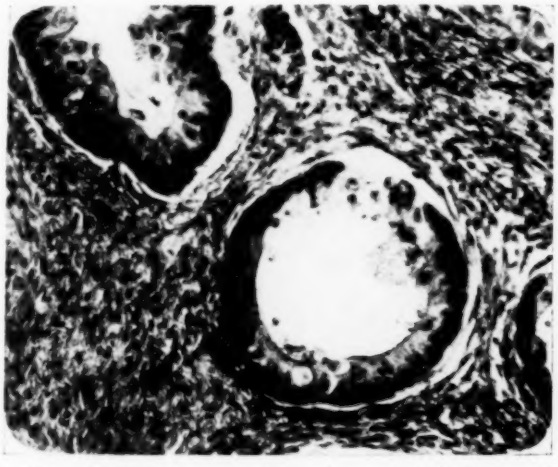


Fig. 15.—Adenocarcinoma, arising from cystadenoma. x250.

center of the fibroma suggested that the growth might have been of congenital origin.

Degenerative changes encountered in the fibromas are of more than passing interest. Hyaline degeneration, necrosis attributable to interference with blood supply, and hemorrhage with liquefaction were encountered with fair frequency as is the case with uterine tumors of similar type. Sarcom-

atous transformation (Fig. 23) was found but once, approximately 5 per cent. A more interesting type of degeneration was observed in three tumors. That the condition is a degeneration and not a tumor type is apparent from the fact that the changes to be described are not found throughout the tumor but only in smaller areas. In the early stages the connective tissue fibrils become swollen and their outlines become vague, while the nuclei stain faintly (Fig. 21). In some areas mucoid degeneration is demonstrable. At a later stage

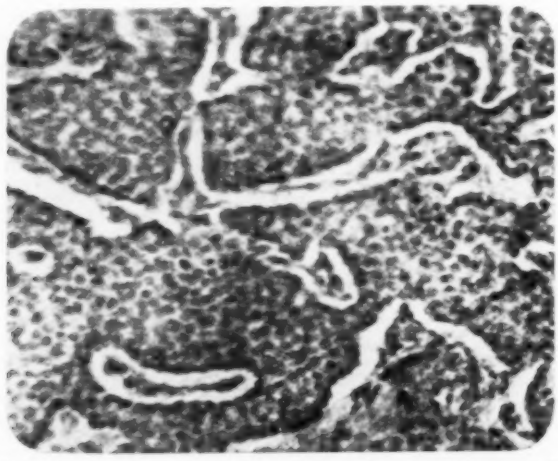


Fig. 16.—Carcinoma, papillary type (nonsecreting). x250.

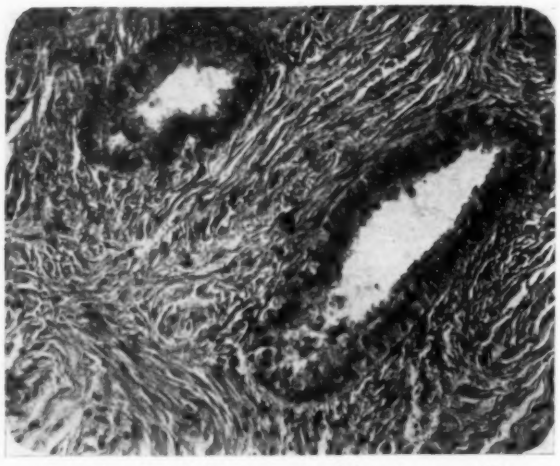


Fig. 17.—Fibroadenoma, glandular type. x250.

the swollen fibrils are transformed into a smooth homogenous matrix of wavy or tubular outline and the again deeply staining nuclei border the hyaline areas (Fig. 22), giving an appearance somewhat similar to the cylindroma of other regions of the body.

Ovarian sarcomas are not frequently observed,—only 15 (3 per cent) being found in the present series. They are usually solid, have a smooth surface, and a varying consisteney. They may arise in the ovary either pri-

marily or secondarily. Scheffy, in 118 cases, found that 60 per cent occurred before the fourteenth year. Histologically the tumors show a great variety in the differentiation and maturity of the cells; the immature cell forms, however, greatly predominate. The spindle-cell variety often appears to be derived from a fibroma, and tumors with this origin often have a relatively benign clinical course.

In the present material there were two main types. In the first, the

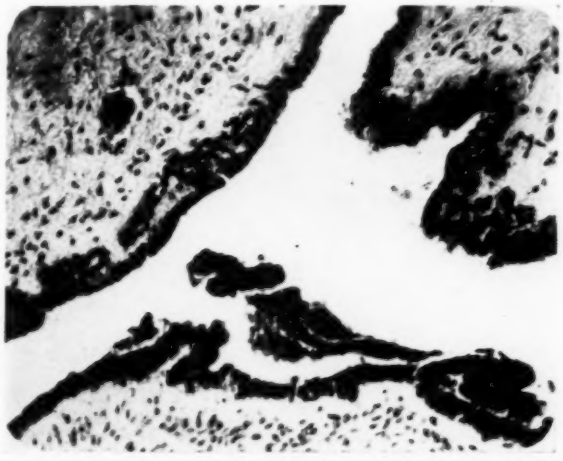


Fig. 18.—Fibroadenoma, nonglandular type. x250.

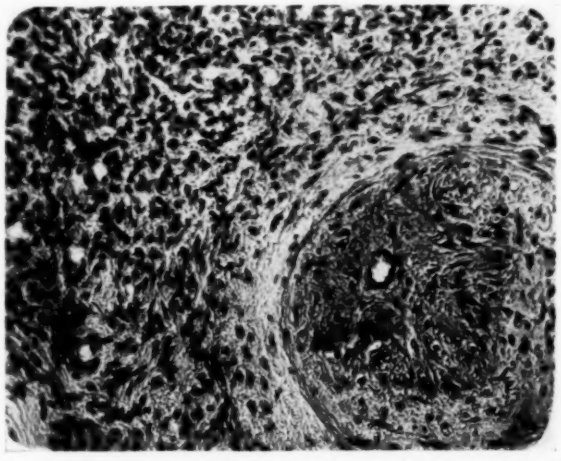


Fig. 19.—Fibroma, arising from wall of blood vessel. x250.

malignant cell was of spindle form; Figs. 24 and 26 illustrate the extremes in this group. The second type was characterized by more or less polyhedral cells with a scattering of single nuclei giant cells in varying number. In these tumors more or less of an alveolar arrangement, which sometimes radiated from blood vessels, was observed.

According to Lippert, carcinomas represent 10.66 per cent of all ovarian tumors and of these 20.75 per cent are, according to Stiebler and Brandess,

of metastatic origin. The tumor may start as a malignant growth or arise secondarily from a previously benign one. Most of the secondary carcinomas have the primary growth situated in the gastrointestinal tract. As with carcinomas in other locations extreme youth is not exempt, and tumors of this type have also been observed in hermaphrodites.

The 64 carcinomas of the present series do not include the teratomas in

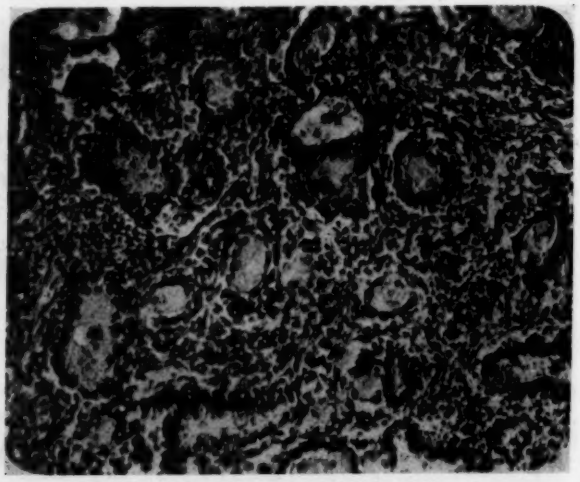


Fig. 20.—Stroma ovarii. x250.

Fig. 21.—Degenerative change in fibroma (early phase). x250.

which malignant degeneration occurred. The neoplasms could be about equally divided in two groups. In the one group the tumor consisted of solid masses of cuboidal, polyhedral, or cylindrical cells arranged about more or less delicate septa. Depending upon the plane of section, the architecture differed from a solid sheet of neoplastic cells to the appearance of Fig. 16, where the structure is of the papillary type. The epithelial cells of this type of tumor showed no evidences of secretory activity.

The second group showed a morphology which indicated the origin from cystadenomas. The stroma was of varying degrees of cellularity and arranged so as to support acini of various shapes and sizes. The acini were lined by many layers of columnar epithelium and the lumen of the alveoli as well as the epithelial cells were more or less distended with the retained products of secretion.

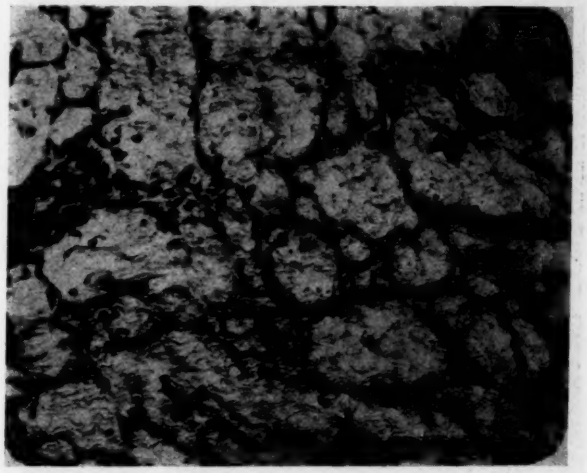


Fig. 22.—Degenerative change in fibroma (late phase). x250.

Fig. 23.—Sarcomatous degeneration of fibroma. x250.

In addition to the two standard types of carcinoma three tumors of the Krukenberg type were found (Fig. 8). Two of these specimens were metastatic, the primary growth of one being in the stomach and of the other in the large intestine. The morphology of both of these as well as of the third case was typical of the type. The stroma was loose and somewhat cellular and scattered throughout this stroma were collections of small acini which were lined by the typical faintly staining signet ring-shaped cells, the individual cells being distended by a secretion of mucoid character.

The third case of Krukenberg tumor occurred in a young girl and, as is usually the case, was bilateral. A second laparotomy, together with a thorough study of the gastrointestinal tract, failed to disclose a primary tumor elsewhere. Death occurred quickly and although no autopsy was obtained the case has been considered as primary in the ovary.

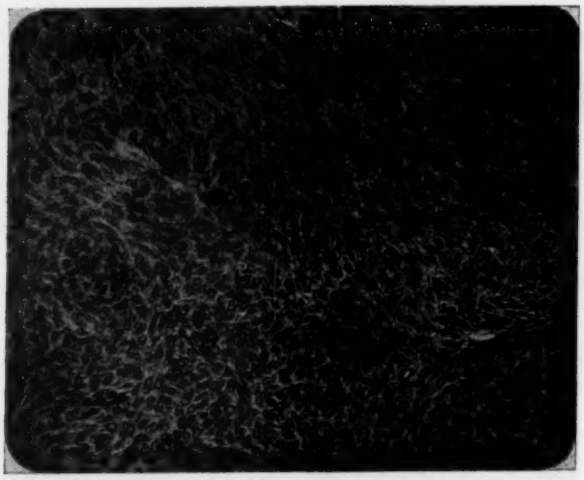


Fig. 24.—Spindle-cell sarcoma with little stroma. x250.

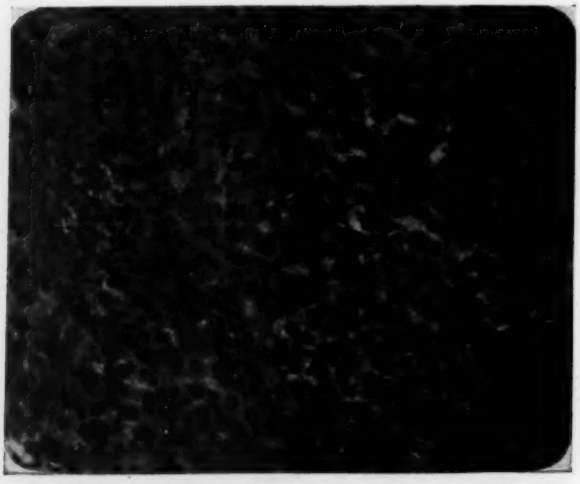
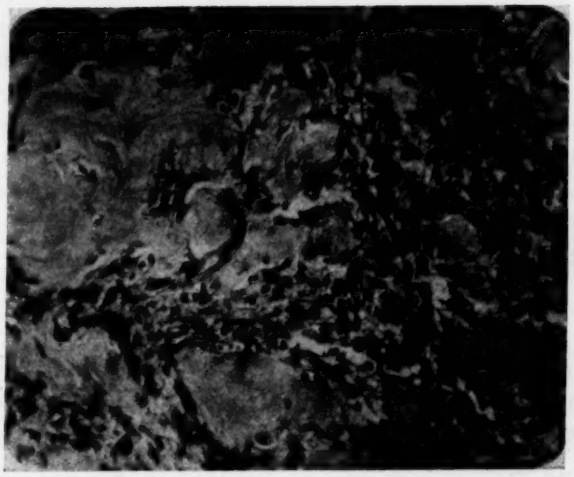


Fig. 25.—Polymorphous cell sarcoma. x250.

TABLE I

HISTOLOGIC CLASSIFICATION	NUMBER	PER CENT OF TOTAL
Adenofibroma	4	0.8
Papilloma	16	3.2
Fibroma	23	4.6
Sarcoma	15	3.0
Teratoma and dermoid cyst	61	12.2
Carcinoma	64	12.8
Cystadenoma	71	14.2
Simple follicle, retention, or corpus luteum cyst	246	49.5



Figs. 26 and 27.—Spindle-cell sarcoma with varying amounts of stroma. x250.

In the series there occurred another bizarre type, one instance of folliculoma ovarii. This has been elsewhere reported by Manheims.

SUMMARY

A series of 500 tumors of the ovary is presented. The incidence of the various types is given in the accompanying table.

EFFECT OF BACILLUS ACIDOPHILUS ON INTESTINAL PUTREFACTION AND INDICAN OUTPUT*

By Max Kahn, M.A., M.D., Ph.D., New York City†

INTRODUCTION

THE decomposition of proteins in the digestive tract is due chiefly to the influence of the gastric, pancreatic, and intestinal proteolytic ferments and, partly also, to the bacteria of the large intestines.

INTESTINAL PUTREFACTION

Intestinal putrefactive processes depend first upon the presence of proteins and second, obviously, upon the presence of putrefactive bacteria, chiefly Bacillus coli communis, Bacillus putrificus, Bacillus aerogenes capsulatus. Changes in the amount of ingested proteins and changes in the virulence of the microorganisms will therefore influence putrefaction. Putrefactive bacteria are facultative, that is, they prefer carbohydrates, if available, to proteins. Thus, the amount of carbohydrates ingested will also be of influence on putrefaction.

THE ETHEREAL SULPHATES

The formation of phenol and phenolic substances (cresol, indol, skatol, etc.) has been ascribed to the action of the intestinal bacterial flora. Such organisms as the Bacillus coli communis, which is a normal inhabitant of the intestinal canal, are harmless under normal circumstances. In conditions of injury to the intestinal mucosa, they may become virulent (Fermi and Salto). Other organisms, like the Bacillus putrificus and Bacillus aerogenes capsulatus, which are obligatory anaerobes, thrive in the colon where there is no oxygen (Herter), and break up protein into carbocyclic toxic substances. These substances are absorbed and, after undergoing oxidative changes, they conjugate with sulphuric acid in the liver producing nontoxic substances, the so-called ethereal sulphates. The toxicity of these bacterial products of putrefaction is well known.

Of the ethereal sulphates, indoxyl potassium sulphate, or indican, has aroused special interest and is regarded as a direct index of the total amount of ethereal sulphates conjugated, and so indicates the extent of putrefactive processes in the intestines. After having been oxidized to indoxyl and after having been conjugated with sulphuric acid and potassium, indol occurs in

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the urine as indoxyl potassium sulphate or indican; and the amount of indican may be taken as an index of the extent of the intestinal putrefaction.

In other investigations, the amount of all of the ethereal sulphates or their relative amount in comparison with the amount of the total sulphur and the percentage of sulphate sulphur, have been studied. Folin showed that indican is that part of the ethereal sulphates which is produced in the process of putrefaction. Another part of the excreted ethereal sulphates is a product of the endogenous sulphur metabolism not derived from ingested proteins.

In support of these facts, it has been shown that low protein diet (Bunge, Corabe), vegetarian (Hoppe-Seyler), farinaceous (Rothman, Gottwald and Krauss), or carbohydrate diet (Hoppe-Seyler, Poehl, Biernacky, etc.), ingestion of lactose (Strauss and Phillipsohn), of milk (Biernacky, Malteoda, etc.), of sour milk (Poehl), and the ingestion of lactic acid bacilli (Cohendy, Leav), of hydrochloric acid (Biernacky, Stadelman, etc.), increase the amount of ethereal sulphates eliminated. It was also found that water drinking (Hawk) and starving (Müller) decrease the amount of ethereal sulphates excreted.

REVIEW OF THE LITERATURE

Since Städeler found phenol in cow's and horse's urine, Landolt, Lieben, Hoppe-Seyler, Buligninsky and Munk found traces of it in normal human urine; and Salkowski observed that in ileus and other obstructive intestinal disease, the excretion of phenol in the urine is much increased.

Baumann and Herter reported that not only phenol, but also other substances were excreted in the urine as ethereal sulphates. They also observed that phenol unites not only with sulphuric acid but with other radicals. This was confirmed by Schmiedeberg, who found that phenol unites with glycuronic acid. Upon poisoning dogs with phenol, it was found that the liver became rich in phenol sulphates. For example, in 100 parts of liver he found 19 times as much tribromphenol as in 100 parts of blood. This phenomenon seemed to prove that the liver is the seat of conjugation of the phenolic and indolic radicals with sulphuric acid.

Lang determined the quantity of ethereal sulphates in the urine of geese before extirpation of the liver. He was led to believe that the synthesis of the ethereal sulphates was not exclusively performed in the liver.

From experiments performed in vitro, Koch also thought that the liver was not the only seat of sulphoconjugation. He took liver, kidney, pancreas, thymus, and muscle, minced each organ separately, and added phenol and disodium sulphate. He kept these mixtures at body temperature or else at 8° to 12° C. He reported that all the tissues, except the thymus, took part in the synthesis. He obtained similar results with ortho-, meta-, and para-dioxyphenol.

Landi repeated the experiments of Koch, using only liver tissue. But, due to the fact that decomposition sets in so very soon, he could not confirm Koch's findings. He also made perfusion experiments with the liver, and

finally assumed that the seat of conjugation of the phenolic and sulphuric radicals was not the liver but the intestines.

The opinion of Lang, Koch, and Landi is directly negated by the findings of Embden and Glaessner. They performed perfusion experiments on the organs of dogs, using the liver, muscle, kidneys, lungs, and small intestines. From their investigations they concluded that the liver is the most important organ for the formation of the ethereal sulphates. Smaller quantities of ethereal sulphates are produced in the lungs and the kidneys, but the muscle tissue and the small intestine play a very insignificant rôle in the formation of the ethereal sulphates.

Beale, from his observations, was of the opinion that the liver was the seat of the synthesis of the ethereal sulphates.

Finizio confirmed Beale from his clinical findings. In normal individuals and in a case of ecchinococcus hepatic cyst, he found that the administration of thymol caused an increased excretion of ethereal sulphates in the urine. When, however, he administered thymol to a patient suffering from hepatic cirrhosis, he found no increase of the ethereal sulphates in the urine.

In normal conditions of the alimentary tract, Strauss and Phillipsohn found no phenol in the urine, and they concluded that, under normal conditions, the phenol and other radicals were conjugated with sulphuric acid. According to these authors, the liver is the seat of the synthesis of the ethereal sulphates.

Herter and Waksman took 7 grams of liver, kidney, muscle, brain, and blood, respectively. These specimens were minced, and each tissue treated with 10 c.c. of a weak phenol solution, and allowed to stand for from two to three hours. The mixtures were then distilled, and they found that there was a loss in the phenol distilled. The liver retained most of the phenol, then came in order, the kidneys, muscle, and brain.

In conditions of jaundice, Biernacki found four times the amount of ethereal sulphates normally present. Darenberg and Percy found an increased excretion of indol and skatol in jaundiced individuals. Labbe and Vitry obtained similar results. Magregeas obtained varying quantities of ethereal sulphates in icteric patients.

EXPERIMENTAL RESEARCH

We undertook the present study to ascertain whether it is possible to decrease the extent of intestinal putrefactive processes by means of influencing the intestinal flora. We used a culture of the Bacillus acidophilus for that purpose *(Vitabac)*, assuming that the increasing of the acidity of the intestinal contents, as produced by the Bacillus acidophilus, will inhibit the growth and virulence of the putrefactive microorganisms.

Bacteriologic examination of the feces was made by Strassburger's method. A small portion of feces with water was centrifuged, the liquid part poured into another tube, diluted with an equal quantity of alcohol, and again centrifuged. From the sediment, which did not now contain anything but bacteria and yeasts, a smear was prepared and stained. The Bacillus acidoph-

ilus was identified by its Gram-positive stain and its morphologic characteristics.

As an index of the extent of the putrefactive processes, we determined the urinary indican sulphur. We also observed whether there occurred a decrease in the urinary indican excretion after the ingestion of the Bacillus acidophilus, and what changes occurred in the urinary output of the ethereal sulphates related to this decreased indicanuria.

We adopted the following technic: The patients were kept on a fixed diet. The urine was collected for two days and analyzed for total sulphur, ethereal sulphates, and indican. The feces were examined bacteriologically. After taking the Bacillus acidophilus culture (we used that prepared by the Bergman Laboratories called *Vitabac*) two tablespoonsful three times daily, the urine was reexamined in three to five days and the feces analyzed as before.

Results.—The total sulphur was determined by Benedict's, the ethereal sulphates by Folin's, and the indican by Ellinger's method. The urine was preserved by cold.

TABLE I

SPONTANEOUS CHANGES IN ETHEREAL SULPHATE AND INDICAN ELIMINATION

CASE NUMBER	TOTAL SULPHUR AS SO_3 IN GRAMS		ETHEREAL SULPHATE SULPHUR AS SO_3 IN GRAMS		INDICAN AS INDIGO IN MILLIGRAMS		ETHEREAL SULPHATE SULPHUR PER CENT OF TOTAL SULPHUR	
	FIRST DETERMINATION	SECOND DETERMINATION	FIRST DETERMINATION	SECOND DETERMINATION	FIRST DETERMINATION	SECOND DETERMINATION	FIRST DETERMINATION	SECOND DETERMINATION
1	1.9	2.7	0.17	0.22			8.7	8.2
2	2.65	2.3	0.15	0.15	2.5	2.5	5.8	3.4
3	4.8	4.8	0.26	0.26	8.9	8.4	5.5	5.5
4	1.75	2.35	0.20	0.23	8.9	18.8	11.4	9.8

TABLE II

ETHEREAL SULPHATE AND INDICAN ELIMINATION BEFORE AND AFTER BACILLUS ACIDOPHILUS ADMINISTRATION

CASE NUMBER	TOTAL SULPHUR AS SO_3 IN GRAMS		ETHEREAL SULPHATE SULPHUR AS SO_3 IN GRAMS		INDICAN AS INDIGO IN MILLIGRAMS		ETHEREAL SULPHATE SULPHUR PER CENT OF TOTAL SULPHUR	
	BEFORE BAC. ACIDOPHILUS	AFTER BAC. ACIDOPHILUS	BEFORE BAC. ACIDOPHILUS	AFTER BAC. ACIDOPHILUS	BEFORE BAC. ACIDOPHILUS	AFTER BAC. ACIDOPHILUS	BEFORE BAC. ACIDOPHILUS	AFTER BAC. ACIDOPHILUS
5	5.6	3.7	0.19	0.22	10.4	4.3	3.4	6.0
6	1.7	1.8	0.17	0.26	10.5	2.4	10.0	14.0
	3.2	6.0	0.32	0.70	32.7	3.4	10.2	11.3
7	3.0					2.8*		
8	3.0	2.2	0.17	0.19			5.6	8.7
9	1.6	2.4	0.09	0.18			5.7	7.4
10	3.4	3.1	0.44	0.56	12.1	8.6	10.0	26.8
	4.6	4.4	0.25	0.30	11.2	7.7	5.5	6.9
11		4.7*		0.47*		1.8*		10.0*
12	2.9	4.4	0.022	0.22	17.0	10.4	0.8	5.0
13	2.7	4.7	0.22	0.25			8.6	9.5
14	2.7	2.05	0.20	0.23	1.3	Traces	7.2	11.2
15	1.1	1.4	0.34	0.34	10.7	11.3	30.0	25.0
16	2.35	2.3	0.47	0.18	19.5	19.4	20.1	7.8
17	1.1	0.7	0.17	0.17	4.6	3.2	15.6	24.1
18	2.65	3.0	0.26	0.33			10.0	11.0
19	1.2	1.2	0.14	0.15			11.2	12.5

*One week later.

Bacteriologic examination of the feces was made in twenty cases. We found that after three to five days, the Bacillus acidophilus appeared in the feces. If given to the patient together with lactose, it became the dominating microorganism of the feces. Such feces appear already physically changed, the odor being less offensive, and a more rapid sedimentation of a greater mass of bacteria could be observed during and after the centrifuging. These improvements in the bacteriology of the feces persisted even two weeks after the patient stopped taking the Bacillus acidophilus culture. Findings similar but less pronounced were obtained when lactose was not given, but in such cases we could observe the appearance of the Bacillus acidophilus in the feces in smaller numbers.

Marked alterations occurred in the urinary indican output (see Table II) in all but two cases. There was a decrease in the indican excreted, ranging from 30 to 91 per cent. This decrease was already present after five days, but gradually became more pronounced if the patient continued taking the Bacillus acidophilus culture (see Table II, Cases 7 and 11). It is obvious that the greatest decrease of the individual output was found in cases where previously the urinary indican elimination and, as it can be assumed, the intestinal putrefaction was more marked.

According to almost all authors, the urinary indican output may be taken as an index of the extent of the putrefactive processes within the intestines. We can, therefore, also conclude from our findings that ingestion of Bacillus acidophilus is likely to diminish intestinal putrefaction.

As to the ethereal sulphates, our findings were unexpected. We found, along with the decrease of the indican excreted, an increase in the percentage of ethereal sulphates (and in the absolute amount) in all but the same two cases in which the decrease of the indican elimination was not obtained (see Table II). The regularity of these findings was definite. The fact that no increase of ethereal sulphates was obtained in two cases in which indican excretion was not deceased suggests a connection between these two changes —that either one is the cause of the other, or that both are the result of an underlying cause. This could only be confirmed by our findings in Case 4, Table I. In that case, the patient was kept on a fixed diet without Bacillus acidophilus to observe the spontaneous changes of the sulphates and of indican. We found an increase in indican output, probably due to an intercurrent enteritis. Along with this increase of indican excretion, a decrease of the percentage of the ethereal sulphates was found. Which of these is the primary change will perhaps be seen by a consideration of Case 15, Table II. In this case, in which there was no decrease in the indican output after the ingestion of Bacillus acidophilus, a carbuncle was present. It is well known that a pus focus in the body goes along with an increased indican production. We may assume in this case that the great part of the indican excretion was of extraintestinal origin. This emphasizes also that there is no increase in the percentage of the ethereal sulphates if indican production is not diminished. It follows from this observation that the decrease of indican produc-

tion is the primary change, inducing by some mechanism, an increase of the percentage of the ethereal sulphates.

Ingestion of Bacillus acidophilus, by decreasing intestinal putrefaction, diminished the percentage of the ethereal sulphates even separate from the factor of decrease of indican production. This is shown by the same case (see Table II). We feel that this effect of the Bacillus acidophilus ingestion—decreasing the percentage of ethereal sulphates—is of intestinal origin, while the increase in the amount of the ethereal sulphates which follows the decrease of the indican formation, may be of an extraintestinal or metabolic origin.

Case 16, in which there was no decrease of indican output, showed a similar behavior. In this case, too, a decrease of the percentage of the ethereal sulphates was found. As these two cases have also a previously high percentage of the ethereal sulphates, the indican production may be assumed, in Case 16, to be due partly to extraintestinal indican formation.

We conclude from these findings that lessened intestinal putrefaction and decrease of indican formation result from ingestion of Bacillus acidophilus and produce a change in metabolism, increasing the percentage of the ethereal sulphates. The ethereal sulphates cannot therefore be taken as an index of intestinal putrefaction, as a secondary quantitative change in the opposite direction may be caused by the lessened indican formation. In Cases 15 and 16, there was no decrease of indican formation; the reduced intestinal putrefaction went along with decreased percentage of ethereal sulphates.

Our control cases, in which the urine and feces were examined at intervals of from eight to fourteen days, show that, without the ingestion of Bacillus acidophilus and without intercurrent diseases, only slight changes occur in the percentage of the ethereal sulphates as well as in the indican output.

Subjectively, ingestion of Bacillus acidophilus produced improvement of the bowel movements, improvement of headache, and a better and stronger feeling generally.

SUMMARY

After ingestion of Bacillus acidophilus for from three to five days, this organism can be found in the feces in large number, if the culture is given to the patient together with lactose; and less in number if given by itself. Along with the appearance of the microorganism in the feces, the urinary indican output decreased in most cases, indicating a decrease in the extent of intestinal putrefaction. There are evidences which seem to show that in the formation of the ethereal sulphates, two opposite effects occur in connection with the Bacillus acidophilus ingestion; a decrease of the "intestinal" ethereal sulphates, caused by the decrease of intestinal putrefactive processes, and an increase of the "metabolic" ethereal sulphates, caused by lessened indican formation. As this would explain the many contradictory findings obtained by different investigators, attention should be paid to these possibilities.

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THE ACTION OF PILOCARPIN ON THE RAT'S PUPIL*

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INTRODUCTION

PILOCARPIN was at one time regarded by many pharmacologists as distinctively a stimulant of the parasympathetic division of the autonomic system, its effect being localized specifically on the myoneural junctions. So firm was this conviction that response to it on the part of an organ or tissue was accepted as evidence of the presence of the above type of innervation. But during approximately the last decade a number of discrepancies in the narrowness of this affinity have been reported, different points of action being exhibited in certain organs of several species of animals. It now seems, as Edmunds[1] has very aptly stated, that no single tissue is attacked by pilocarpin to the exclusion of all others.

These vagaries on the part of pilocarpin have recently been reviewed by Edmunds[1] and by Sollmann.[2] For convenience, the most important of them may be briefly cited here as follows: (A) The parasympathetic myoneural junctions in most of the organs of the usual laboratory animals; (B) the sympathetic myoneural junctions in the uterus of the rat (Gunn and Gunn[3]); (C) the muscle substance of the bladder (Edmunds and Roth[4]) and the retractor penis (Edmunds[5]); and (D) the cervical sympathetic ganglia (Dale and Laidlaw[6]). In all the above, pilocarpin manifests uniformly a stimulant action, so that they are examples of transference of the point of action without alteration of the direction of action. Furthermore, in each of these cases, atropine was antagonistic and hence followed philocarpin in the transposition.

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Up to the present time there has been presented but one instance of dissociation of the antagonism of pilocarpin and atropine (intestine of the frog, Roth[7]), and but one of an apparent inversion of pilocarpin to a depressant (cardiac vagus of the frog, Langley[8]). The latter may, however, be explained as a stimulation of the sympathetic accelerator (Sollmann[2]). In the former, physostigmine was also transposed and dissociated from atropine antagonism. But, while pilocarpin is transposed on the frog's rectum, arecolin exhibits its usual parasympathetic action (Schuller[9]). Physostigmine, on the other hand, is the one transposed in the case of the turtle's intestine, pilocarpin and atropine maintaining their affinity for the parasympathetic (Roth[10]).

As the title of this article indicates, this investigation is concerned with the action of pilocarpin solely on the iris of the rat. It is of interest chiefly in the presentation of an example of the caprice of this alkaloid. It may be here stated, in brief, that pilocarpin produces in the rat a mydriasis, which is antagonized by physostigmine and arecolin but not by atropine; hence, it would seem to be an instance of inversion of the action of a parasympathetic stimulant or of its transposition to the sympathetic apparatus.

MATERIALS AND METHODS

Animals.—The subjects observed were healthy albinos, bred in my laboratory. All ages from six months to two years were employed. Before beginning an experiment, the animals were examined for light reflex and equality of pupils.

Fig. 1.—Diagram of thread counter's glass fitted with 0.5 mm. scale for measuring the size of the rat's pupil. *A*, the lens; *B*, the base with the aperture which is placed over the orbit.

Pilocarpin.—The hydrochloride was the salt studied. The following brands were examined: Merck, Eimer and Amend, Sharp and Dohme, and Parke, Davis and Co. All produced identical effects.

Administration.—Solutions of the hydrochloride were administered in the following ways: (*A*) Locally, into the conjunctival sac, in concentrations of from ⅛ per cent to 8 per cent; (*B*) subcutaneously into the flank, 1.5 mg. to 4 mg. per kg.; and (*C*) intrahepatically (Waddell[11]), 2.5 mg. per kg.

Examination of Pupils.—A thread counter's glass was employed. To the base of this a half mm. scale was attached, so that it could be brought very close to the pupil and the size be read off on the magnified scale. Since the lens had a very narrow focal range, readings accurate to 0.1 mm. could be taken. The examinations were made in diffuse daylight; the light reflex was studied in direct sunlight and under a Zeiss "hammer" lamp.

Rat's Pupils.—The rat's pupils are round and exceedingly small, measuring in diffuse daylight from 0.5 mm. to 1 mm. and in direct sunlight from 0.25 mm. to 0.5 mm. While they respond readily to changes in illumination, they are not subject to such fluctuations as are observed in the cat and the rabbit due to handling and the movements of the observer.

EXPERIMENTAL DATA

The data will be considered under two captions: (A) The Pilocarpin Mydriasis, and (B) The Influence of Other Drugs and Procedures.

A. The Pilocarpin Mydriasis.—Dilatation of the pupil followed pilocarpin by whatever route administered, locally, subcutaneously, and intrahepatically. Miosis was never exhibited at any stage of the experiment. Since most of the data were obtained by application to the conjunctival sac, the others being merely confirmatory, details will be given only for the local action.

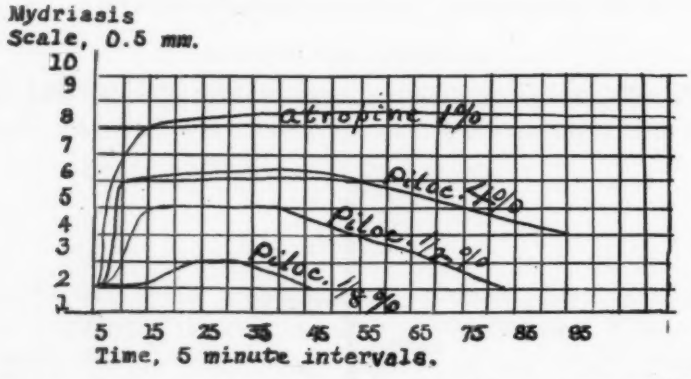

Fig. 2.—Curves, showing the average degree and time relations of the mydriasis after different concentrations of pilocarping and after 1 per cent atropine.

Concentrations of from ⅛ per cent to 8 per cent were all effective in producing mydriasis. There was a progressive increase in the degree, the rapidity of production, and the duration of the dilatation on increasing the strength of the solution up to 4 per cent; above that no difference was detectable. For instance, ¼ per cent dilated a 0.5 mm. pupil to 1 mm. and a 4 per cent to 2.5 mm., while an 8 per cent gave only 2.5 mm.

The pilocarpin mydriasis was never maximal. Atropine and asphyxia markedly increased the size of the pilocarpin pupil, as did also epinephrin and cocaine. It may be further noted here that the latter two drugs did not effect in the rat a maximal dilatation and that atropine and asphyxia also enhanced their mydriasis.

Dropped into the conjunctival sac, the lower concentrations produced no effect in the untreated (other) eye, but those of 2 per cent to 8 per cent gave a tardy and slight dilatation (absorption with resulting systemic action). Other effects were not observed in the rat, the drug apparently causing the animal no discomfort whatever. Even large doses subcutaneously did not impair the desire to eat.

Exophthalmos was appreciable with the higher concentrations of the drug but not after the lower. In cases where careful measurements were made, the widening of the palpebral fissure was by as much as 1 mm. The degree exhibited after epinephrin was marked and out of all proportion to that following pilocarpine.

No abnormalities in the position of the eyeball were noted. There was a slight photophobia, and the upper lid was perceptibly lowered in strong lights.

The pilocarpin pupil, when the full effect of the drug had been elicited, did not constrict to light, but the initial stages of its mydriasis could be thus opposed. As the effect of the drug was wearing off, however, the light reflex was restored; for instance, a previously dilated pupil that had receded to 1 mm. could be constricted to its normal of 0.5 mm.

There is then exhibited in the rat after administration of pilocarpin a submaximal mydriasis which is not antagonized by light except in its initial and recessional stages. The phenomenon is due to a peripheral action of the drug and is accompanied by a slight degree of exophthalmos.

B. The Effect of Other Drugs and Procedures.—As an aid in localizing the point of action of pilocarpin on the rat's iris, the behavior of the pupil to other drugs and procedures was determined. The results and their bearing on the pilocarpin mydriasis may be briefly stated as follows:

Sodium Chloride.—Solutions of 1 per cent, 2 per cent, and 4 per cent produced no effects. These were usually employed in the control eye during the study of the action of drugs on the other.

Carbon Dioxide.—Carbon dioxide produced initially a constriction, often pinpoint in degree; dilatation was not observed until a marked degree of asphyxia had been reached—usually respiratory and general paralysis—at which time the pupil suddenly and maximally dilated with loss of the light reflex. The miosis was prevented by pilocarpin, but the mydriasis was unaffected by either the prior or subsequent administration of the drug.

Epinephrin.—Applied locally, epinephrin did not dilate the rat's pupil; but injected intrahepatically there followed instantaneously a marked degree of exophthalmos and a submaximal mydriasis with loss of light reflex. The dilatation was the same in degree as was observed after pilocarpin. The two drugs exhibited mutual summation. Extreme degrees of asphyxia, however, effected even a greater degree of mydriasis than pilocarpin and epinephrin combined.

Chloral Hydrate.—Chloral hydrate, 0.3 gm. per kg., rectally, produced a pinpoint pupil, a phenomenon previously described in man and animals[12] and ascribed to central action. This miosis can be prevented and removed by pilocarpin applied locally.

Atropine.—Atropine as dilute as 0.1 per cent produced so great a degree of dilatation that only a microscopic rim of iris remained visible. There was the usual loss of the light reflex. Due to the extreme degree of mydriasis, further effects could not be detected after pilocarpin, cocaine, and other mydriatics.

Homatropine.—This drug affected a maximal dilatation with loss of the light reflex. The latter observation is at variance with the results reported on other animals.[13] On the rat, it corresponded in every way with atropine.

PROTOCOL No. 1

Pupil Time	Rat No. 27 Poisoned	Weight, 150 gm. Pupil—Size in 0.5 mm.	Pilocarpin—Atropine Nonpoisoned
12:30	1.0		1.0
Pilocarpin 4 per cent	--	Saline 4 per cent	--
12:31	1.0		1.0
12:32	1.0		1.0
12:33	1.0		1.0
12:34	2.0		1.0
12:39	3.0		1.0
12:41	4.0 light		1.0
12:45	5.0 reflex		2.0
12:46	5.0 lost		2.0
Atropine 1 per cent	--	Saline 1 per cent	--
12:48	6.0		2.0
12:54	7.0		4.0
12:55	8.0		6.0
12:56	8.0		7.0

Protocol No. 1.—Attention is called to the submaximal dilatation, the tardy effect on the nonpoisoned pupil, and the loss of the light reflex after pilocarpin, and to the great increase in the pilocarpin mydriasis after atropine.

PROTOCOL No. 2

Pupil Time	Rat No. 17 Poisoned	Weight, 250 gm. Pupil—Size in 0.5 mm.	Pilocarpin—Cocaine Nonpoisoned
10:32	1.5		1.5
Cocaine 2 per cent	--	Saline 2 per cent	--
10:34	2.0		1.5
10:35	2.5		1.5
10:37	4.0		1.5
10:40	4.0		1.5
Pilocarpin 2 per cent	--	Saline 2 per cent	--
10:50	6.0		1.5
Pilocarpin 4 per cent	--	Saline 4 per cent	--
11:00	6.0		2.0
Atropine 1 per cent	--	Saline 1 per cent	--
11:15	9.0		6.0

Protocol No. 2.—Summation of the effects of pilocarpin and cocaine is shown.

PROTOCOL No. 3

Pupil Time	Rat No. 35 Poisoned	Weight, 175 gm. Pupil—Size in 0.5 mm.	Pilocarpin—Arecolin Nonpoisoned
12:40	1.0		1.0
Arecolin 2 per cent	--	Saline 2 per cent	--
12:45	0.2		1.0
12:48	pinpoint		1.0
12:50	pinpoint		1.0
Pilocarpin 4 per cent	--	Saline 4 per cent	--
12:55	0.5		1.0
12:56	1.0		2.0
11:00	3.0		3.0
Arecolin 2 per cent	--		--
11:15	1.0		1.0

Protocol No. 3.—The mutual antagonism between pilocarpin and arecolin is shown.

Cocaine.—Introduced into the conjunctival sac, a submaximal dilatation was produced. It was like that after pilocarpin and epinepherin, with which it was additive. Atropine and asphyxia, extended the cocaine mydriasis to a maximal.

Physostigmine.—One drop of a 2 per cent physostigmine solution gave a pinpoint pupil within two minutes. The untreated eye exhibited the same phenomenon after five minutes, by which time well marked systemic effects were in evidence. This drug and pilocarpin were mutually antagonistic on the pupil, but not systemically so.

Arecolin.—This alkaloid produced effects on the pupil identical with those following physostigmine. No systemic effects were observed on local application.

Morphine.—No effect was exhibited prior to the onset of restlessness and dyspnea, at which time there was shown a dilatation (probably asphyxia). After lethal doses, a maximal dilatation like that after carbon dioxide was in evidence. In view of the above, no interaction with pilocarpin could be studied.

Ergotoxin.—The results with this drug were inconstant and ambiguous on both local and intrahepatic administration. Pilocarpin produced its usual mydriasis at ten, thirty, and sixty minute intervals after ergotoxin.

DISCUSSION

As a preliminary, it may be stated that the rat's pupil reacts to light, asphyxia, chloral, epinephrin, physostigmine, arecolin, cocaine, and atropine in a conventional manner. Accordingly, it would seem that its iris is innervated like those of other experimental animals—a constrictor mechanism with a parasympathetic nerve and a dilator with a sympathetic.

Pilocarpin applied to the conjunctival sac of one eye produces a mydriasis, initially, in that eye alone. Binocular dilatation is exhibited only after high concentrations of the drug but even then very tardily as compared with the monocular. The point of action is, accordingly, at the periphery, the phenomenon not being due to an indirect effect from the adrenals or asphyxia.

To further localize the point of action, the evidence given by other drugs and procedures must be analyzed. A peripheral dilatation could be produced, obviously, by (*A*) stimulation of the dilator muscle or nerve with or without depression of the constrictor apparatus, (*B*) simultaneous but unequal stimulation of both constrictor and dilator mechanisms, or (*C*) depression of the constrictor nerve or muscle. These possibilities will be considered in the order mentioned.

(*A*) Relative to the effect being due to a stimulation of the dilator structures, the following may be noted: (1) the similarity of the mydriasis by pilocarpin, cocaine, and epinephrin; (2) the summation of its action with those of cocaine and epinephrin; (3) the accompanying exophthalmos; and (4) the fact that other examples of similar transposition are known. But this

evidence is not conclusive, in that it does not explain the abolition of the light reflex and of the chloral miosis. If there were, moreover, a simultaneous depression of the constrictor, a maximal mydriasis should have been exhibited, the imbalance being in such a case all in favor of dilatation. Since the observations were to the contrary, this hypothesis does not seem tenable.

(*B*) Simultaneous stimulation of the dilator and constrictor mechanisms would explain all the phenomena, provided there be granted a greater degree of action on the dilator than on the constrictor and provided the two opposing receptors were always affected to the proper relative degree at the same instant. It is improbable that this could occur. Should, on the other hand, pilocarpin attack: (*a*) the constrictor sooner than the dilator, an initial miosis would have been in evidence; or (*b*) the dilator before the constrictor, a dilatation followed by a constriction would have been exhibited. But continuous observation failed to disclose such phenomena during the course of the pupillary action of pilocarpin; hence, the mydriasis does not seem to be due to a simultaneous stimulation of both structures.

(*C*) The evidence for a depression of the constrictor apparatus is as follows: (*a*) the blocking of stimuli from the constrictor center, demonstrated by abolition of the light reflex and the chloral miosis; (*b*) the agreement in direction of action with atropine and carbon dioxide, which remove the tonic constrictor impulses; (*c*) the antagonism to physostigmin and arecolin. The incompleteness of the pilocarpin mydriasis may be explained as indicating a weaker action than atropine, reducing but not entirely abolishing the effect of the tonically acting center; while atropine and extreme degrees of asphyxia entirely nullify the influence of the central stimuli, the former by paralyzing the nerves and the latter the center. Furthermore, the mutual antagonism between pilocarpin and physostigmin and arecolin is indicative of nervous rather than muscular depression, hence, the pilocarpin mydriasis must be attributed to a depression at the periphery of the parasympathetic nerves.

SUMMARY

1. The rat's pupil exhibits physiologically and pharmacologically conventional effects, except to pilocarpin; hence, the innervation appears to be the same as in other animals.

2. Pilocarpin dilates the rat's pupil on both local and systemic application.

3. The pilocarpin mydriasis is a peripheral phenomenon in the rat, since it is limited to the eye to which the drug is applied.

4. In the rat, pilocarpin abolishes the light reflex, the initial constriction of asphyxia, and the miosis of chloral, and hence blocks central parasympathetic impulses.

5. Pilocarpin antagonizes the effects of physostigmine and arecolin on the rat's pupil; accordingly, it affects the nervous rather than the muscular elements.

6. Pilocarpin on the rat's pupil acts like atropin but less potently.

CONCLUSIONS

Pilocarpin dilates the rat's pupil by depressing the parasympathetic constrictor apparatus at the periphery.

Pilocarpin, in its effect on the rat's pupil, presents an example of inversion of the direction of action without transposition of the point of action.

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STUDIES IN BLOOD GLYCOLYSIS*

General Consideration of Glycolysis in Relation to the Blood Cells, and the Production of Lactic Acid and Carbon Dioxide

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A REVIEW of the literature on glycolysis warrants the conclusion that the sugar of shed blood diminishes on standing, independently of bacterial contamination. Some observers[1] have stated that the sugar of diabetic blood decreases less rapidly than that of normal blood. But whether the less rapid glycolysis in diabetes is due to a different type of glucose[2] in the circulating blood is still a debatable question. A number of other possible factors may be responsible for this alleged difference between diabetic and nondiabetic bloods. As a preliminary to the study of the rôle played by the erythrocytes and leucocytes in carbohydrate metabolism, it seemed advisable to study the relation of the blood cells to glycolysis in vitro. Since these studies were begun (early in 1923), several reports have appeared in the literature on somewhat analogous experiments. Although some of the results presented in this communication are not entirely new, it is believed that they are of sufficient interest to warrant their publication. The phenomenon of glycolysis has been studied in animal and human bloods, and in all analyses the Folin-Wu method[3] was adopted for blood sugar determinations. Potassium oxalate was employed as an anticoagulant for the whole blood. The amount of the potassium oxalate used was roughly 0.06 per cent of the blood. It appeared important to con-

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trol this factor since Aibara,[4] Macleod,[5] and Hiller, Linder and Van Slyke[6] have reported that potassium oxalate inhibits glycolysis.

In Tables I *a* and I *b* are recorded the results of studies of glycolysis in the blood of rabbits and oxen. The blood was drawn in all instances from the veins under aseptic conditions and maintained at 38° C. free from bacterial contamination. Analyses of the bloods for sugar were made immediately and after three, six, and twenty-four hours. The oxen blood was re-

TABLE I *a*

BLOOD GLYCOLYSIS ON RABBIT*

		SUGAR											
		OXALATED BLOOD			CO SATURATED			SERUM			LAKED WITH H_2O		
NO	TEST HOUR		LOSS IN			LOSS IN			LOSS IN			LOSS IN	
		MG.	MG.	%	MG.	MG.	%	MG.	MG.	%	MG.	MG.	%
1.	At once	139			140			150			139		
	3 hours	111	28	20	114	26	19	150	0	0	139	0	0
	6 hours	100	39	28	103	37	26	149	1	0	139	0	0
	24 hours							150	0	0			
2.	At once	132			129			163			132		
	3 hours	103	29	22	115	14	11	163	0	0	132	0	0
	6 hours	97	35	27	98	31	24	163	0	0	132	0	0
	24 hours	70	62		76	53	41	163	0	0	132	0	0

*Specimens incubated at 38° C. under aseptic conditions.

TABLE I *b*

GLYCOLYSIS ON OX BLOOD IN VITRO—AT 38° C.

		OXALATED BLOOD			DEFIBRINATED BLOOD			SERUM		
OX NO.	DURATION OF TEST	SUGAR	LOSS IN		SUGAR	LOSS IN		SUGAR	LOSS IN	
		MG.	MG.	%	MG.	MG.	%	MG.	MG.	%
1.	30 min.	88			88			125		
	4 hours	70	18	20	75	13	15	125	0	0
	20 hours							124	1	1
2.	30 min.	91			91			119		
	4 hours	81	10	10	83	8	9	115	4	3
	20 hours	60	31	33	73	18	20	115	4	3
3.	30 min.	94			94			115		
	4 hours	85	9	10	92	2	2	115	0	0
	20 hours	72	22	23	77	17	18	115	0	0
4.	30 min.	100						125		
	4 hours	86	14	14				125	0	0
	20 hours	79	21	21						
5.	30 min.	90						134		
	4 hours	84	6	7				134	0	0
	20 hours	65	25	28				134	0	0
6.	30 min.	88								
	4 hours	73	15	17						
	20 hours	58	30	34						
7.	30 min.	125						187		
	4 hours	100	25	20				187	0	0
	20 hours	94	31	25				187	0	0
8.	30 min.	88						120		
	4 hours	71	17	19				120	0	0
	20 hours	70	18	20				120	0	0
9.	30 min.	89								
	4 hours	71	18	20						
	20 hours	41	48	54						

ceived into sterile containers when the animals were slaughtered and the sugar was determined as soon as the blood reached the laboratory (20 min.) after four hours and after twenty hours. Changes in the blood sugar of the rabbits have been observed in the oxalated whole blood, in the whole blood saturated with carbon monoxide, in the blood serum, and in blood laked by dilution with water. To saturate the blood with carbon monoxide, the gas was slowly bubbled through the blood until the maximum absorption was obtained. Laking was effected by adding one volume of blood to seven volumes of water. Results are reported in milligrams of sugar per 100 c.c. of blood at the time of analy-

TABLE II

BLOOD GLYCOLYSIS IN VITRO AT ROOM TEMPERATURE

CASE	AGE	SEX	TEST SPECIMEN	DURATION OF TEST				DIAGNOSIS
				At once mg.	3 hours mg.	6 hours mg.	24 hours mg.	
1. N. G.	50	M	Whole blood	105	91	80	22	Angina pectoris
			Serum	110	110	110	110	
			Laked blood	105	105	105	105	
2. J. P.	45	M	Whole blood	242	220	200		Diabetes
			Serum	277	277	268	268	
			Laked blood	242	242	242	242	
3. J. M.	59	M	Whole blood	176	166	154		Diabetes
			Plasma	185	185	183		
			Laked blood	176	176	176		
			CO saturated	170	166	154		
			R+ washed b. c.	128	125	121		
4. M. G.	61	M	Whole blood	105	92	78	43	Cardiac insufficiency
			Plasma	111	110	110	110	
			Laked blood	105	105	105	105	
			CO saturated	105	94	83	52	
			R+ washed b. c.	187	176	176	151	
5. A. A.	46	M	Whole blood	105	92	81	42	Traumatic neuritis
			Plasma	107	107	107	108	
			Laked blood	105	105	105	104	
			R+ washed b. c.	187	179	173	150	
6. F. C.	58	M	Whole blood	124	101	90		Chronic myocarditis
			Plasma	129	129	128	128	
			Laked blood	124	124	123	124	
			R+ washed b. c.	189	178	176	124	
7. T. S.	25	F	Whole blood	91	77	71	36	Hyperthyroidism
			Plasma	92	92	92	92	
			Laked blood	91	91	91	91	
			R+ washed b. c.	192	172	167	144	
8. W. G.	47	M	Whole blood	95	79	64		Teratoma testicle
			Plasma	110	109	108		
			Laked blood	95	94	95		
			CO saturated	95	78	63		
			R+ washed b. c.	200	197	190		
			R+ plasma	189	188	188		
9. H. M.	45	M	Whole blood	98	79	63		Lymphosarcoma
			Plasma	103	101	102	102	
			Laked blood	98	99	99		
			R+ washed b. c.	194	189	174		
			R+ plasma	182	181	181	179	

Case 3—Blood corpuscles washed with 0.9 per cent saline solution twice.

Case 4, 5, 6, 7, 8, and 9 corpuscles washed with Ringer's solution with 0.2 per cent glucose twice.

R = Ringer's solution with 0.2 per cent glucose.

sis, and the loss of sugar is recorded in milligrams per 100 c.c. and as a percentage of the initial figure. During a period of six hours at 38° C. the whole blood of the rabbit suffers a loss of about 27 per cent of its sugar, and after twenty-four hours about 47 per cent. Analogous changes in the blood saturated with carbon monoxide were observed. Kawashima[7] has also demonstrated that carbon monoxide is without influence on glycolysis. There is, however, no loss of sugar in the blood serum or in the laked blood. Glycolysis was observed in oxen blood; here, however, the disappearance of the sugar was less rapid. In 8 of the 9 cases after twenty hours at 38° C. the loss of sugar amounted to 20 to 34 per cent of the original concentration. In the last experiment a loss of 54 per cent after twenty hours was noted. In the defibrinated oxen blood a fall of sugar similar to the oxalated blood was seen, but in the serum the sugar remained unchanged. The apparent fall of 1 per cent in Case 1 and 3 per cent in Case 2 is no doubt due to analytical errors. This fall in blood sugar is not due to activity of microorganisms, since the specimens were cultured at the end of incubation and gave no bacterial growth.

Table II presents data on glycolysis in human blood. Nine cases were studied, and of these 2 were diabetics, 1 a hyperthyroid, and the remainder miscellaneous cases with no evident disturbance of carbohydrate metabolism. In all instances a continuous decrease of the sugar of the whole oxalated blood is observed after three, six, and twenty-four hours at room temperature, but the sugar of the serum, plasma and laked blood remain unchanged. In Cases 3, 4, and 8 the drop in the sugar of the blood saturated with carbon monoxide parallels that of the oxalated blood. From these observations on rabbit, oxen and human bloods, the conclusion appears warranted that the presence of the intact blood cells is essential for glycolysis. To substantiate this opinion, in Case 3 the erythrocytes were removed by fractional centrifugation in narrow tubes and washed with 0.9 per cent NaCl solution. The red cells were then suspended in four times their volume of Ringer's solution containing 0.2 per cent of Kahlbaum's glucose. This mixture was allowed to stand for twenty-four hours at room temperature, and determinations of the blood sugar were made at intervals of three, six, and twenty-four hours. A drop in the sugar concentration of the mixture was found, but the decrease is less than that observed in the whole blood. In Cases 4, 5, 6, 7, 8, and 9 similar experiments were conducted except that the blood cells were washed with the Ringer's solution containing the glucose in place of the 0.9 per cent NaCl. A decrease of the sugar was found in all instances; however, the loss of sugar is less than that reported for the corresponding whole bloods. In 8 and 9 the glycolytic action of the plasma towards the sugar contained in the Ringer's solution was examined. In these 2 experiments the volume of plasma used corresponded to the volume of the blood cells in the former experiments. No change was noted in the concentration of the sugar of the mixture after standing three, six, or twenty-four hours.

A comparison of the glycolytic action of the blood cells upon the blood sugar as controls, and upon the sugar added to Ringer's solution has been made in 8 cases, of whom 3 were diabetics. The results of these experiments are

recorded in Table III. The whole oxalated blood was allowed to stand at 38° C. under aseptic conditions for twenty-four hours. Determinations of the sugar were made immediately and at three, six, and twenty-four hour intervals. A fall in blood sugar is observed in all cases. As in the previous experiments the erythrocytes of the corresponding bloods were separated by centrifugation, washed with physiologic salt solution and suspended in 4 times their volume of Ringer's solution containing 0.2 per cent glucose. These mixtures were analyzed for sugar, simultaneously with the whole bloods. The hemoglobin concentrations of the whole bloods are reported as indices of the cell volumes. In the final column the loss of sugar in milligrams per 100 c.c. is recorded. The sugar concentration is diminished both in the whole bloods and in the mixtures of erythrocytes and Ringer's solution. In Case 8, the whole blood and the erythrocytes in Ringer's solution were maintained for twenty-four hours under anaerobic conditions. In this instance the whole blood during twenty-four hours under aerobic conditions lost 70 mg., and during the same period under anaerobic condition lost 65 mg. per 100 c.c.. The loss in sugar in the mixture of erythrocytes and Ringer's solution is comparable with losses in the other cases under aerobic conditions. The loss of sugar in these mixtures cannot be explained by the glycolysis of the sugar contained in the red cells alone. The maximum concentration of sugar in the erythrocytes was found to be 0.024 per cent. Since the cells formed but one-fifth of the total volume of the mixtures, it is evident that if all the sugar of the erythrocytes disappeared, the loss would not exceed 5 mg. per 100 c.c. The sugar loss, however, varies from 25 to 39 mg., hence it is obvious that a portion of the glucose of the Ringer's solution no longer reacts as glucose. In the nondiabetic bloods the loss of sugar is not greater than that of the diabetics. Of the three diabetics, Case 4 had received insulin but Cases 3 and 5 had received no insulin before drawing the blood. It is observed that the rate of glycolysis in Cases 3 and 5 is greater than that seen in 4. There appears to be no demonstrable difference in the rate of glycolysis in either diabetic or hyperthyroid and normal bloods. Macleod,[7] Macleod and Pearce,[8] and Cajori and Crouter[9] have been able to demonstrate no significant variation in the glycolysis of diabetic and nondiabetic bloods. It is also interesting to note that the blood cells of diabetic bloods when incubated with Ringer's solution containing glucose produce a loss of sugar as great as that observed with nondiabetic blood cells. A quantitative comparison of the glycolytic action of the erythrocytes in the whole blood and in Ringer's solution cannot be made from these data since no attempt was made to preserve the same volume of erythrocytes in the Ringer's solution as that found in the corresponding whole bloods.

A comparison of the rate of glycolysis effected by the blood cells in their plasma and in Ringer's solution containing glucose has been made in Table IV. The blood cells were washed once with physiologic salt solution and then added to 4 times their volume of Ringer's solution containing 0.2 per cent glucose, and in four times their volume of plasma. The volume of cell suspension used was adjusted so that the cell count was about 2.3 million per cu. mm. and the leucocyte count about 220 per cu. mm. Sugar concentrations were determined

TABLE III

BLOOD GLYCOLYSIS IN VITRO ON WHOLE BLOOD AND WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION WITH 0.2 PER CENT GLUCOSE AT 38° C.

CASE	AGE	SEX	TEST SPECIMEN	DURATION OF TEST: At once mg.	3 hours mg.	6 hours mg.	24 hours mg.	Loss in mg.	Hb. gm. per 100 c.c.	DIAGNOSIS
1. E. P.	19	F	Whole blood	65	49	35	17	32	10.3	Hyperthyroidism
			R + B. C.	170	156	150	117	39		
2. C. C.	21	F	Whole b'ood	87	66	41	18	48		Auricular fibrillation
			R + B. C.	170	159	153	122	37		
3. R. E.	60	F	Whole blood	176	158	125	49	109	11.3	Diabetes
			R + B. C.	172	155	150	127	28		
4. P. G.	41	F	Whole blood	125	91	70	28	63	12.6	Diabetes
			R + B. C.	172	156	148	124	32		
5. E. B.	62	F	Whole blood	185	163	138	72	91	13.2	Diabetes
			R + B. C.	170	155	148	123	32		
6. M. P.	50	F	Whole blood	105	74	50	25	49	8.3	Carcinoma cervix
			R + B. C.	170	158	151	132	26		
7. M. M.	35	F	Whole blood	90	70	52	26	44	12.7	Fibroid of uterus
			R + B. C.	172	159	153	134	25		
8. G. R.	50	M	Whole blood	134	106	71	36	70	14.5	Alkalosis
			Anaerobic	135	114	84	49	65		CO_2C.P. = 92
			R + B. C.	169	161	154	135	26		$P_H = 7.6$

R + B. C. = Washed blood corpuscles in Ringer's solution with 0.2 per cent glucose.
Hb = Hemoglobin.
Case 3 and 5—no insulin treatment.
Case 4—insulin treatment.
Blood corpuscles washed once with 0.9 per cent saline solution.
Sugar content in washed blood corpuscles = 0.024 per cent.

immediately and after twenty-four hours. The Ringer's solution had a P_H of 7.35. The loss of sugar from the Ringer's solution in all instances was greater than that from the plasma; however, the initial sugar concentration was higher in the Ringer's solution than in the plasma. Continuous washing of the blood cells with 0.9 per cent sodium chloride solution diminishes the rate of glycolysis when these cells are added to Ringer's solution containing glucose. It is seen in Table V *a* that the amount of sugar lost during twenty-four hours incubation at 38° C., progressively diminishes with the number of washings of the blood cells in physiologic salt solution.

The rate of glycolysis appears dependent upon the number of blood cells present in the incubated mixture. In Table V *b* there is a comparison of the

TABLE IV

GLYCOLYSIS ON WASHED BLOOD CORPUSCLES IN PLASMA AND IN RINGER'S SOLUTIONS CONTAINING 0.2 PER CENT GLUCOSE INCUBATED AT 38° C.

CASE	DURATION OF TEST	1 C.C. WASHED BLOOD CORPUSCLES ADDED TO 4 C.C. RINGER'S SOLUTION		1 C.C. WASHED BLOOD CORPUSCLES ADDED TO 4 C.C. PLASMA	
		mg.	Loss in mg.	mg.	Loss in mg.
1. L. C.	At once	176		80	
	24 hours	121	55	42	38
2. D. T.	At once	182		97	
	24 hours	146	36	67	30
3. C. G.	At once	176		91	
	24 hours	129	47	51	40
4. A. M.	At once	174		63	
	24 hours	128	46	30	33
5. R. R.	At once	176		100	
	24 hours	129	47	67	33
6. I. R.	At once	172		63	
	24 hours	129	43	40	23
7. C. H.	At once	170		83	
	24 hours	125	45	53	30
8. M. M.	At once	172		135	
	24 hours	120	52	97	38

Ringer's solution $P_H = 7.35$.
Blood corpuscles washed once with 0.9 per cent NaCl solution.
Blood corpuscles suspended both in Ringer's solution and in plasma.
Erythrocytes average 2,300,000 per cu. mm.
Leucocytes average 220 per cu. mm.

rate of glycolysis in mixtures in which the washed blood cells were suspended in 4 times and in 1.5 times their volume of Ringer's solution containing glucose. In the former mixtures the erythrocytes averaged 2.3 million and the leucocytes 220 per cu. mm., while in the latter mixtures the erythrocytes were about 4.5 million and the leucocytes 500 per cu. mm. In the latter mixtures, the initial sugar concentration is less than in the former, but the amount of sugar lost within twenty-four hours is more than double that lost in the mixtures containing the smaller number of blood cells. Macleod[10] concludes from his numerous experiments on glycolysis that it is an intracorpuscular process. Levine and Meyer[11] have shown that when leucocytes were suspended in Henderson's phosphate solutions containing glucose, a portion of the glucose, as such, disappeared with the production of lactic acid. It is difficult to say in the experiments re-

TABLE V *a*

INFLUENCE OF WASHING AND CENTRIFUGING OF BLOOD CORPUSCLES UPON GLYCOLYSIS—STANDING AT 38° C.

CASE*	DURATION OF TEST	ONCE WASHED BLOOD CORPUSCLES 1 C.C. + 4 C.C. RINGER'S SOL. WITH 0.2 PER CENT GLUCOSE		4 TIMES WASHED BLOOD CORPUSCLES 1 C.C. + 4 C.C. RINGER'S SOL. WITH 0.2 PER CENT GLUCOSE		ONCE WASHED BLOOD CORPUSCLES 1 C.C. + 4 C.C. PLASMA		4 TIMES WASHED CORPUSCLES 1 C.C. + 4 C.C. PLASMA	
		mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.
1. L. C.	At once	176		169		80		79	
	24 hours	121	55	135	34	42	38	50	29
2. D. T.	At once	182		182		97			
	24 hours	146	36	160	22	67	30		
3. R. R.	At once	176		172		100		103	
	24 hours	129	47	140	32	67	33	81	22

*Washed blood corpuscles added to the same patients' plasma.

ported in the Table V *b* whether the glycolysis is due principally to the erythrocytes or the leucocytes. From the observations of Osnato and Killian[12] on the lactic acid of the cerebrospinal fluid in meningitis it appears that this phenomenon may play an important part in carbohydrate metabolism, particularly in infections.

TABLE V *b*

GLYCOLYSIS ON WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION CONTAINING 0.2 PER CENT GLUCOSE IN DIFFERENT COMBINATIONS—INCUBATED AT 38° C.

CASE	DURATION OF TEST	1 C.C. WASHED BLOOD CORPUSCLES ADDED TO 4 C.C. RINGER'S SOLUTION		2 C.C. WASHED BLOOD CORPUSCLES ADDED TO 3 C.C. RINGER'S SOLUTION	
		Sugar		Sugar	
		mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.
1. C. G.	At once	176		133	
	24 hours	129	47	24	109
2. A. M.	At once	174		135	
	24 hours	128	46	26	109
3. R. R.	At once	176		150	
	24 hours	129	47	50	100
4. I. R.	At once	172		138	
	24 hours	129	43	46	92
5. C. H.	At once	170		134	
	24 hours	125	45	28	106

4 c.c. Ringer's solution + 1 c.c. washed blood corpuscles.
R.B.C. average = 2,300,000 per cu. mm.
W.B.C. average = 220 per cu. mm.
3 c.c. Ringer's solution + 2 c.c. washed blood corpuscles.
R.B.C. average = 4,500,000.
W.B.C. average = 500 per cu. mm.

TABLE VI

GLYCOLYSIS ON WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION CONTAINING GLUCOSE, FRUCTOSE, AND GALACTOSE—INCUBATED AT 38° C.

CASE	DURATION OF TEST	GLUCOSE 1 c.c. once washed B. C. added to 4 c.c. Ringer's solution + 0.2 per cent glucose		FRUCTOSE 1 c.c. washed B. C. added to 4 c.c. Ringer's solution		GALACTOSE 1 c.c. washed B. C. added to 4 c.c. Ringer's solution	
		Sugar		Sugar		Sugar	
		mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.
1. L. C.	At once	176		176		128	
	24 hours	121	55	134	42	107	21
2. D. T.	At once	182		174		147	
	24 hours	146	36	146	28	130	17
3. R. R.	At once	176		175		133	
	24 hours	129	47	154	21	113	20

The loss of sugar during incubation with blood cells is not confined to glucose. A comparison of the rate of sugar loss in incubated mixtures of blood cells containing glucose, fructose, and galactose has been made in Table VI. The loss of sugar expressed as milligrams per 100 c.c. after twenty-four hours' incubation at 38° C. is greatest with glucose and less with fructose and galactose.

Specimens of whole blood from 5 individuals, 2 cases of hyperthyroidism and 3 of diabetes, were divided into 3 groups. One group of specimens was maintained in hot air incubator at 38° C.; the second group was permitted to stand at room temperature (20°-22° C.); and the third was placed in the ice box at 4° C. for twenty-four hours. The sugar concentrations of all specimens were determined at intervals of three, six, and twenty-four hours. Table VII gives the data on the sugar concentrations found, and the loss in sugar expressed in milligrams per 100 c.c. and in per cent of the original figure. For all three periods of incubation the maximum loss occurs in the blood maintained at 38° C. and the minimum in those preserved in the ice box. At a temperature of 38° C. the loss in sugar after twenty-four hours expressed as per cent of the original concentration varied from 42 to 83 per cent. at room temperature from 23 to 78 per cent and in the ice box from 2 to 33 per cent. The rate of glycolysis appears to vary directly with the temperature to 38° C. It is of practical importance to note that preserving blood in the ice box does not prevent a loss of sugar.

A comparison of glycolysis before and after the production of a hyperglycemia by the ingestion of glucose has been made in the experiments recorded in Table VIII. It is seen that after twenty-four hours at 38° C. in Case 1 the blood has lost 92 mg. per 100 c.c. of sugar, this loss amounting to 83 per cent of the original concentration. The ingested glucose produced a blood sugar of 195 mg. per 100 c.c. During twenty-four hours, 155 mg. of sugar for every 100 c.c. are lost from this blood, representing 79 per cent of the original concentration. In the hyperglycemic blood the loss expressed in milligrams per 100 c.c. is greater than in the control blood; but expressed as a fraction of the original amount of sugar, it is equivalent to that of the control. In the second case the intake of glucose has raised the blood sugar from 93 to 120 mg. per 100 c.c. In both specimens of blood 80 mg. of sugar per 100 c.c. of blood are lost after twenty-four hours at 38° C. The loss after the glucose ingestion expressed as per cent of the original concentration is less than that of the control blood.

The addition of insulin to whole blood during the period of incubation was without effect upon the rate of glycolysis. It is seen in Table IX that the loss of sugar at 38° C. in the specimens of blood to which insulin had been added (10 units per 10 c.c. blood) parallels the loss of sugar in the control specimens.

Evans[13] has observed a fall in the carbon dioxide capacity of shed blood which, he believes, is due to a conversion of glucose into lactic acid as a result of glycolysis. Kraske[14] in his studies on seven human subjects reported a quantitative conversion of the lost blood sugar into lactic acid during an incubation of two hours. Kondo,[15] however, in his experiments on glycolysis in dogs' blood during a similar period of incubation found that the increase in lactic acid was much less than the amount of sugar lost. Von Noorden[16] has produced evidence to show that the lactic acid formed is d-lactic acid. Morgulis and Barkus[17] have attempted to emphasize the difference between glycolysis in vitro and the glycolysis within the organism after insulin on the basis of their observations that in the former the disappearance of the sugar goes parallel with a formation of lactic acid. Their figures, however, do not demonstrate a parallel rise in lactic

TABLE VII

BLOOD GLYCOLYSIS IN VITRO AT DIFFERENT TEMPERATURES

CASE‡	AGE	SEX	DURATION OF TEST	38° C. INCUBATED			ROOM TEMPERATURE			ICE BOX			DIAGNOSIS
				Sugar mg.	Loss in mg.	Loss in per cent	Sugar mg.	Loss in mg.	Loss in per cent	Sugar mg.	Loss in mg.	Loss in per cent	
1. R. R.	28	F	At once	91			91			91			Hyperthyroidism
			3 hours	61	30	33	72	19	21	89	2	2	
			6 hours	40	51	56	64	27	30	88	3	3	
			24 hours	17	74	81	20	71	78	78	13	14	
2. J. E.	25	F	At once	110			110			†110			Hyperthyroidism
			3 hours	81	29	26	89	21	19	99	11	10	
			6 hours	56	54	49	76	34	31	93	17	15	
			9 hours	36	74	67	55	55	50	88	22	20	
			24 hours	18	92	83	40	70	64	74	36	33	
3. P. G.	41	F	At once	434			434			434			Diabetes*
			3 hours	400	34	8	417	17	4	422	12	3	
			6 hours	370	64	15	406	28	6	417	17	4	
			9 hours	341	93	22	400	34	8	416	18	5	
			24 hours	251	183	42	334	100	23	410	24	6	
4. A. G.	42	F	At once	300			300			300			Diabetes
			3 hours	261	39	13	261	39	13	291	9	3	
			6 hours	252	48	16	254	46	15	291	9	3	
			24 hours	166	134	45	230	70	23	238	62	21	
5. E. G.	63	F	At once	176			176			176			Diabetes*
			3 hours	138	38	22	153	23	13	174	2	1	
			6 hours	131	45	26	144	32	18	174	2	1	
			24 hours	76	100	57	105	71	40	172	4	2	

*Insulin treatment.

†Ice box disordered (not enough cold).

‡Specimens cultured after standing twenty-four hours; no bacterial growth.

TABLE VIII

BLOOD GLYCOLYSIS IN VITRO AT 38° C. COMPARE FASTING BLOOD WITH ONE HOUR AFTER 100 GM. GLUCOSE INGESTION

CASE	AGE	SEX	DURATION OF TEST	SUGAR						HEMOGLOBIN	
				Before glucose			After glucose			Before glucose	After glucose
				mg.	Loss in mg.	per cent	mg.	Loss in mg.	per cent	mg.	mg.
1. J. E.	25	F	At once	110			195				
			3 hours	81	29	26	151	44	22		
			6 hours	56	54	49	130	65	33		
			9 hours	36	74	67	88	107	55		
			24 hours	18	92	83	40	155	79		
2. H. F.	12	M	At once	93			120				
			3 hours	69	24	26	97	23	19		
			6 hours	45	48	52	75	45	37		
			24 hours	13	80	86	40	80	67		
3. E. N.	32	M	At once	94			167			13.3	13.3
			6 hours	68	26		141	26			
			24 hours	20	74		103	64			
4. R. B.	34	F	At once	89			165			12.2	12.1
			6 hours	53	36		140	25			
			24 hours	21	68		85	80			
5. C. G.	16	F	At once	95			227			13.5	13.3
			6 hours	54	41		195	32			
			24 hours	10	85		161	66			
6. N. S.	18	F	At once	114			153			14.2	13.9
			6 hours	63	51		115	38			
			24 hours	14	100		55	98			

TABLE IX

GLYCOLYSIS ON OXALATED BLOOD WITHOUT AND WITH INSULIN

CASE	TEST HOUR	SUGAR			
		OXALATED BLOOD WITHOUT INSULIN		OXALATED BLOOD WITH INSULIN	
		mg. per 100 c.c.	Loss in mg.	mg. per 100 c.c.	Loss in mg.
1. Rabbit	At once	166		166	
	3 hours	142	24	145	21
	6 hours	110	56	112	54
2. Rabbit	At once	155		155	
	3 hours	137	18	137	18
	6 hours	110	45	115	40
3. C. G.	At once	95		95	
	6 hours	54	41	63	32
	24 hours	10	85	14	81
4. N. S.	At once	114		113	
	6 hours	63	51	71	42
	24 hours	14	100	17	96

0.5 c.c. insulin (10 units) added to 10 c.c. blood.
0.1 per cent cresol 0.5 c.c. added to 10 c.c. control blood.
Incubated at 38° C.

acid. Further, Briggs, Koechig, Doisy and Weber,[18] Tolstoi, Loebel, Levine, and Richardson,[19] and Katayama and Killian[20] have reported increases in the lactic acid content of the blood following insulin administration to dogs and human subjects. In the 5 experiments reported in Table X the changes in the lactic acid content of the blood have been studied in relation to the sugar loss during glycolysis at 38° C. and at room temperature under aerobic conditions.

TABLE X

BLOOD GLYCOLYSIS AND LACTIC ACID FORMATION*

CASE	AGE	SEX	DURATION OF TEST	BLOOD SUGAR mg.	Loss in mg.	LACTIC ACID mg.	Rise in mg.	DIAGNOSIS
1. F. S.	26	M	At once	99		16.9		Dementia precox
			3 hours	70	29	18.0	1.1	
			6 hours	52	47	19.1	2.2	
			24 hours	20	79	21.4	4.5	
2. N. G.	50	M	At once	105		16.2		Angina pectoris
			3 hours	91	14	18.9	2.7	
			6 hours	80	25	19.8	3.6	
			24 hours	22	83	33.3	17.1	
3. J. P.	45	M	At once	242		18.9		Diabetes
			3 hours	220	22	25.2	6.3	
			6 hours	200	42	27.9	9.0	
			24 hours	59†	183	29.3	10.4	
4. D. S.	48	M	At once	82		17.0		Normal
			3 hours	71		17.6		
			6 hours	52		20.1		
			24 hours	13	69	20.8	3.8	
5. E. F.	4	M	At once	81		15.7		Normal
			3 hours	64		19.4		
			6 hours	38		24.5		
			24 hours	13	68	28.9	13.2	

*In experiments 1, 4, and 5 blood kept in incubator at 38° C.; in remaining experiments bloods kept at room temperature.

†Bacterial contamination.

TABLE XI

BLOOD GLYCOLYSIS AND CO_2 COMBINING POWER OF PLASMA OF SHED BLOOD ON STANDING AT 38° C.

CASE	AGE	SEX	ANALYZED SUBJECT	TEST HOUR At once	4 hours	24 hours	LOSS IN VOLUME 4 hours	24 hours
1. H. S.	24	M	Sugar (mg.)	124	79	31	45	93
			CO_2C.P. (vol. per cent)	61.4	51.3	37.3	10.1	24.1
2. B. S.	32	F	Sugar (mg.)	110	71	18	39	92
			CO_2C.P. (vol. per cent)	61.7	50.4	40.9	11.3	20.8
3. I. H.	8	F	Sugar (mg.)	165	118	66	47	99
			CO_2C.P. (vol. per cent)	55.1	46.6	37.2	8.5	17.9
4. L. W.	27	M	Sugar (mg.)	113	82	41	31	72
			CO_2C.P. (vol. per cent)	58.9	52.2	37.2	6.7	21.7
5. B. G.	30	F	Sugar (mg.)	197	153	86	44	111
			CO_2C.P. (vol. per cent)	60.7	54.1	39.0	6.6	21.7

Specimen divided into three sterilized centrifuge tubes (about 6 c.c. blood in each tube) and kept in incubator.

Clausen's method[21] with the permanganate oxidation has been employed for the lactic acid determinations. The increase in lactic acid during twenty-four hours is only from 10 to 50 per cent of the amount of sugar lost. In all instances there was observed a rise in the lactic acid; however, the increase is not as great as that reported by Morgulis and Barkus[17] for sheep and dog blood.

The effect of glycolysis on the alkaline reserve of the blood plasma is seen from Table XI. During twenty-four hours incubation at 38° C. there is noted a continuous decrease in the carbon dioxide combining power of the blood plasma, accompanying the drop in sugar. After four hours incubation the fall in the carbon dioxide capacity of the plasma varies from 6 to 10 volumes per

TABLE XII

CHANGING OF SUGAR AND CO_2 CONTENT OF SHED BLOOD ON STANDING IN INCUBATOR AT 38° C. UNDER ANAEROBIC CONDITIONS

CASE	AGE	SEX	SPECIMENS	TEST HOUR At once	TEST HOUR 24 hours	LOSS IN VOLUME
1. G. I.	38	F	Blood sugar (mg.)	183	66	117
			CO_2 content of plasma (vol. per cent)	55.0	53.0	2
2. J. P.	16	M	Blood sugar (mg.)	103	38	65
			CO_2 content of plasma (vol. per cent)	63.3	60.0	3.3
3. H. R.	15	F	Blood sugar (mg.)	157	71	86
			CO_2 content of plasma (vol. per cent)	65.0	55.1	9.9
4. M. K.	26	M	Blood sugar (mg.)	189	85	104
			CO_2 content of plasma (vol. per cent)	55.1	53.2	1.9
5. M. A.	31	F	Blood sugar (mg.)	136	44	92
			CO_2 content of plasma (vol. per cent)	56.6	52.8	3.8
6. Y. K.	52	F	Blood sugar (mg.)	306	142	164
			CO_2 content of plasma (vol. per cent)	58.5	54.7	3.8
7. H. S.	24	M	Blood sugar (mg.)	104	24	80
			CO_2 content of plasma (vol. per cent)	59.1	50.7	8.4
8. W. M.	8	M	Blood sugar (mg.)	114	18	96
			CO_2 content of plasma (vol. per cent)	57.2	48.8	8.4

TABLE XIII

CHANGES IN GLUCOSE AND GAS CONTENT ON WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION AND PHYSIOLOGIC SALT SOLUTION CONTAINING GLUCOSE UNDER ANAEROBIC CONDITIONS AT 38° C.

CASE	TEST SPECIMENS	SUGAR At once	SUGAR 24 hours after	SUGAR Loss	VOL. OF GAS At once	VOL. OF GAS 24 hours after	ERYTHROCYTES million per cu. mm.	LEUCOCYTES per cu. mm.
1. F. M.	Blood corp. in R. + 0.2 per cent glucose	160	112	38	1.2	1.4	2.3	120
	Blood corp. in R. + 0.1 per cent glucose	85	53	32	1.3	1.3	1.8	310
	Blood corp. in 0.9 per cent NaCl sol. + 0.2 per cent glucose	165	135	30	1.3	1.3	1.8	430
2. E. N.	Blood corp. in R. + 0.2 per cent glucose	156	118	38	1.1	1.1	2.3	210
	Blood corp. in 0.9 per cent NaCl sol. + 0.2 per cent glucose	154	133	21	1.1	1.1	2.4	300
3. R. B.	Blood corp. in R. + 0.2 per cent glucose	158	135	23	1.1	1.2	2.3	180
	Blood corp. in 0.9 per cent NaCl sol. + 0.2 per cent glucose	161	135	26	1.0	1.0	2.3	330
4. Rabbit	Blood corp. in R. + 0.2 per cent glucose	170	114	56	1.2	1.4	2.5	180
	Blood corp. in 0.9 per cent NaCl sol. + 0.2 per cent glucose	176	121	55	1.1	1.1	2.5	120
5. Rabbit	Blood corp. in R. + 0.2 per cent glucose	167	115	52	1.1	1.3	2.3	200

Total volume = 10 c.c. each test.
Sugar expressed in mg. per 100 c.c.

cent, and after twenty-four hours from 17.9 to 24.1 volumes per cent. The production of a nonvolatile acid must be responsible for this depletion in the alkaline reserve although Mellanby and Thomas[22] believe that the fall in the alkali reserve of the blood plasma during glycolysis is due to the lactic acid produced. The amount of lactic acid formed during glycolysis (Table X) is sufficient to account for but a small fraction of the decrease in alkaline reserve noted in Table XI. Evans[13] has shown that this decrease in the carbon dioxide capacity of the shed blood proceeds with a progressively diminishing velocity. From the figures reported in Table XI, it is evident that from ⅓ to ½ of the total decrease occurs within the first four hours of incubation. It appears then that other fixed acids in addition to lactic acid must be formed during glycolysis.

During anaerobic glycolysis at 38° C. for twenty-four hours there is little change in the carbon dioxide content of the blood plasma (Table XII). In the 8 experiments tabulated, the blood sugar decrease varied from 65 to 164 mg.; the greatest decrease was observed in the specimen showing a hyperglycemia (306 mg.) immediately after withdrawal. The fall in carbon dioxide content ranged from 1.9 to 8.4 volumes per cent. This decrease in the carbon dioxide content may be explained by a loss of the gas into the oil at the temperature of incubation. Association of the data in Tables X and XI demonstrate that the carbon dioxide, released from the plasma bicarbonate during the neutralization of the acids produced in glycolysis, is retained in the oil-covered blood. Apparently glycolysis does not result in a production of carbon dioxide from the glucose lost.

The experiments reported in Table XIII were planned to obtain further information on this point. Blood cells washed once with 0.9 per cent NaCl solution were placed in Ringer's solution containing 0.1 or 0.2 per cent glucose. In all cases the total volume of the mixture was 10 c.c. and incubation was carried out under anaerobic conditions. A similar loss of sugar is noted in the suspensions of blood cells in Ringer's solution and in physiologic salts solution. It is of interest to note, also, that in Experiment 1 the loss in sugar from the Ringer's solution containing 0.1 per cent glucose is 3.2 mg., whereas the loss from solution containing 0.2 per cent glucose was 3.8 mg. The proportion of cells to the solutions was adjusted so that the erythrocyte content of all mixtures would be fairly close. The erythrocyte content varied from 1.8 to 2.5 million per cu. mm., and the leucocytes from 120 to 430 per cu. mm. The total volume of gas in the mixtures before and after incubation appear unchanged. The volume of gas was determined on the centrifuged specimens by following the technic for CO_2 content. The volumes of the gas were measured at from 20° to 25° C., and although these volumes have not been corrected to reduce them to volumes of CO_2, it is evident that since the volumes remained practically unchanged, no production of CO_2 occurs as a result of glycolysis.

SUMMARY

The sugar of shed blood gradually decreases on standing without bacterial contamination and under either aerobic or anaerobic conditions. This decrease is greatest at 38° C. and least in the ice box.

Plasma, serum and hemolysed blood show no loss of sugar on standing.

Saturation of whole blood with carbon monoxide does not inhibit glycolysis.

When washed blood cells are added to Ringer's solution or to physiologic salt solution containing glucose, fructose or galactose, glycolysis occurs. The decrease in sugar concentration is greatest with glucose and least with galactose.

The rate of glycolysis is dependent upon the blood cell volume.

There appears to be no demonstrable difference between the rates of glycolysis in diabetic and nondiabetic bloods. Furthermore the blood cells of diabetic bloods cause as rapid a rate of glycolysis in Ringer's solution as the cells of nondiabetic bloods.

Insulin therapy or insulin in vitro has no effect upon the rate of glycolysis.

The decrease in sugar concentration is accompanied by a production of lactic acid, but the increase in lactic acid does not account for the total amount of sugar lost.

Other acids than lactic acid are evidently produced during glycolysis. There is no production of carbon dioxide.

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A NOTE UPON THE PRESERVATION OF SHEEP CELLS FOR USE IN COMPLEMENT-FIXATION TESTS*

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WHILE freshly collected cells are to be preferred for the conduct of complement-fixation tests it frequently becomes necessary, under certain circumstances or combinations of circumstances, to resort to some method of preserving blood for varying periods.

This contingency may confront small laboratories or even large ones in which the keeping of laboratory sheep is impracticable for one reason or another. Abattoir blood, for example, may not be obtainable at regular or unvarying intervals, or as has happened, foot and mouth disease or other epidemic condition may interfere with the source or regularity of the supply.

In common with many other workers, for reasons beyond immediate control, I have used abattoir blood for the preparation of cell suspensions for complement-fixation tests, and in order to lessen the frequency of collection the blood has been preserved. Such emergencies, as for example quarantine or embargo, have occurred in the past, which, without a satisfactory method of preservation, would have occasioned some annoyance and great inconvenience.

The method of preservation adopted and in use for some years is neither original nor new. It has been so satisfactory, however, that in view of the number of methods which have been proposed (thus arguing for imperfections in all), attention is again called to it in this note.

There are several details essential for success. Unless the possibility is recognized and due precautions are taken, blood may be collected in pails or other abattoir containers and transferred to the laboratory jars as called for, and, as may happen, when the blood so collected stands for some time, or perhaps, because the container may be hastily or carelessly cleaned, the blood on its arrival in the laboratory will not be fit either for immediate use or for preservation.

The procedure followed is given here in detail. An ordinary quart size Mason jar is thoroughly cleaned and sterilized by dry heat. A sufficient quantity of 10 per cent sterile sodium citrate in normal saline is added to make a layer about one inch deep in the jar. The jar and its contents are then autoclaved.

One of these jars, by previous arrangement, is kept at the abattoir and *filled directly from the animal* when the sheep is killed, and a new container is left in its place when the blood is collected.

Immediately upon arriving in the laboratory the blood is preserved by the

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following method: To each 80 c.c. of blood is added 1 c.c. of a 1:10 dilution of formalin in normal saline after the method described by Bernstein and Kaliski.[1]

A convenient quantity—240 c.c. or 320 c.c.—of the preserved blood is placed in a clean, dry, glass-stoppered bottle which is kept in the ice chest.

Cells are perfectly satisfactory for use for a period of two weeks after preservation by this method. While it is the custom in this laboratory to collect blood at weekly intervals, blood has been preserved and used in emergency for as long as twenty-seven days.

According to Kolmer,[2] the blood is not considered fit for use unless the following conditions are fulfilled:

1. Absence of discoloration of the supernatant fluid after the second washing with normal saline.

2. Return of the normal bright red color on washing.

Blood so collected and preserved has been found satisfactory, in emergency, for the preparation of blood plates.

The method is simple, rapid, inexpensive, eminently satisfactory, and deserves a wide circulation.

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THE INFLUENCE OF DIET ON THE PHYSIOLOGIC ASSAY OF INSULIN*

By Dr. A. Stasiak (Budapest)

VARIOUS authors have investigated the effect of diet on the susceptibility of animals to insulin. Macleod and coworkers[1] observed that the blood sugar in starved rabbits did not recover as rapidly as in fed rabbits, following the hypoglycemia due to insulin. Page[2] showed that rabbits on a diet of oats and bread, which produces slight acidosis, are very resistant to insulin. These results were confirmed by Blatherwick and coworkers,[3] who found that rabbits kept on a diet poor in carbohydrates were more resistant to insulin than were animals well fed with carbohydrates.

Tütso[4] found that the initial fall in blood sugar after insulin injection in rabbits was more pronounced after carbohydrate feeding than after prolonged starvation. Abderhalden and Wertheimer,[5] using white rats, also found these animals most susceptible to insulin on a diet rich in carbohydrates.

The following experiments were performed in order to investigate the

*From the Insulin Committee Laboratory, University of Toronto, Toronto, Canada. Received for publication, May 2, 1926.

influence of diet on the results of the physiologic assay of insulin as ordinarily conducted.

Rabbits were well fed with carrots, hay, and oats, the food being removed fifteen minutes before the commencement of the experiments. As controls, rabbits which had been starved for twenty-four hours were used. All the animals were injected with the same dose of the same insulin (designated Standard S.6), the dose being 2 units per 2 kg. body weight. Blood samples were taken before injection and one and a half, three, and five hours after injection. The blood sugar was determined by the method of Shaffer and Hartmann,[6] and the assays were calculated by the formula given by Macleod and Orr.[7]

The average lowering of the blood sugar ("a" in the formula) was 0.033 per cent in the case of the 18 fed animals used, and 0.031 per cent in the

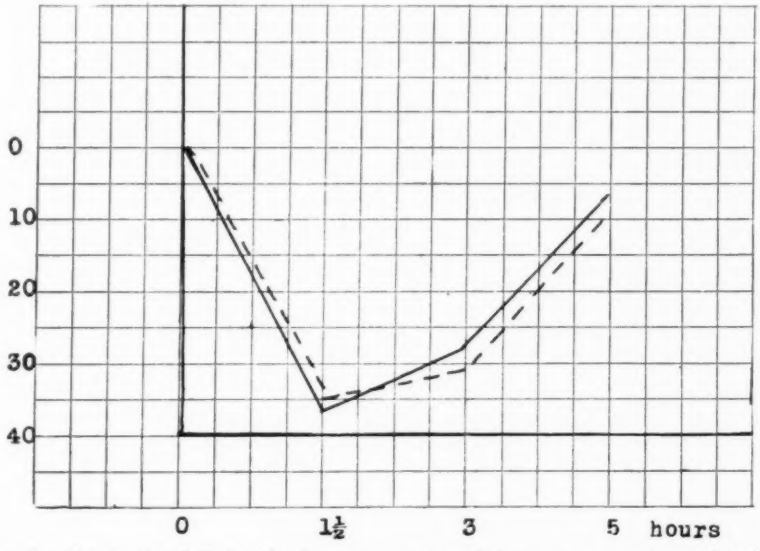

Fig. 1.—Shows the fall in blood sugar, expressed in mg. per cent, produced by the injection of 2 units per 2 kg., and observed at the times shown. The solid line represents the fall in fed animals, and the broken line the fall in starved animals.

case of the 18 starved animals, the corresponding assay results being 10.2 units per c.c. and 12.1 units per c.c., respectively.

In the accompanying table we give the average values of the normal blood sugar and of the blood sugar one and a half, three, and five hours after the injection of insulin. From these figures we have calculated the percentage fall of the blood sugar at one and a half, three, and five hours in relation to the normal blood sugar.

The tabulated results are plotted in the figure. A few observations were also undertaken to study the effect of fasting and carbohydrate feeding on the initial fall in blood sugar following insulin. The striking increased sensitiveness of carbohydrate-fed animals, described by Tütso and others, was not observed to occur. This may have been due to the occurrence of a temporary increase in blood sugar in the fasting animals immediately following the injection of insulin.

TABLE I

	NORMAL %	1½ HOURS %	3 HOURS %	5 HOURS %	"a"	ASSAY IN UNITS
Average for 18 fed animals:	0.139	0.088	0.102	0.130	0.033	10.2
Percentage fall in blood sugar	0	37	28	7		
Average for 18 starved animals:	0.120	0.077	0.083	0.109	0.031	12.1
Percentage fall in blood sugar	0	36	31	9		

CONCLUSION

It would rather appear, from the above results, that the actual range through which the blood sugar is lowered by insulin is approximately the same in fed rabbits and in rabbits from which food has been withheld for twenty-four hours.

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A NOTE ON THE COMPLEMENT-FIXATION REACTION IN ANTIPOLLEN SERUM*

By Susan Griffith Ramsdell, M.A., Los Angeles, Calif.

WHETHER the allergic states in man have a basis in common with the condition resulting from artificial immunization in animals may still be a debatable question. But success in demonstrating certain definite immunologic phenomena—precipitins, complement fixation, shock, skin reaction, Schultz-Dale reaction,—following treatment of laboratory animals with pollens or pollen extracts, has been reported many times. The failures to confirm the findings and the scarcity of experimental data in many of the reports have, however, left enough shadow of doubt upon the status of the test in this connection to seem to warrant a report, with a description in some detail of the special difficulties involved, of another effort to secure complement fixation in the sera of rabbits treated with pollen.

In this laboratory the greatest single need was for a standard method of preparation, stabilization, and measuring the antigenic content of the solutions of pollens with which patients were to be treated for the purpose of desensitization. As to the reported methods of preparation, each offered a particular advantage: one that the protein extraction was more complete; another that the danger of bacterial and other decomposition was more reduced, etc. The most commonly accepted standard was that of the nitrogen content. We wished to find a method where, as far as possible, the process of standardization would be biologic rather than chemical, as representing more nearly what happens in the nonexperimental (allergic) conditions. Complement fixation in pollen-treated rabbits had been reported with enough success to seem to justify its use.

Artemisia californica, corresponding to ragweed in the East in the frequency with which it caused hay fever, was chosen. This was made up in solution by a formula which, on the practicable side, had been the most satisfactory of the many used.

Antigen I. Dried pollen, ether extracted forty-eight hours in a mixture of	5.65 gm.
Sodium bicarbonate, 1.25%	50.00 c.c.
U. S. P. glycerine	50.00 c.c.

This gave the following analysis:

Nitrogen	0.5 mg. per c.c.
Protein nitrogen	0.25 mg. per c.c.
Protein	1.50 mg. c.c.

Pollen units 50,000 per c.c. (One P.U. being 10^{-6} gm. dry, ash-free pollen, by definition.

*From the Clinic of George Piness, Los Angeles. With acknowledgment of direction from Dr. Hyman Miller and of aid in chemical procedures from Dr. Gordon Alles.
Received for publication, May 1, 1926.

TABLE I

RABBIT	TREATMENT				COMPLEMENT FIXATION
	DATE	DOSAGE	ROUTE	INTERVAL	RESULTS
1	10/28/24	2.5 c.c. Antigen II	Intravenously	Tested 4 times in 8 weeks	All negative
2	10/28/24	5.0 c.c. Antigen II	Intraperitoneally	3 weeks	Negative
				5 weeks	Positive
3	10/28/24	2.5 c.c. Antigen II	Intravenously	3 weeks	Negative
				5 weeks	Positive
4	11/21/24	Gradual Antigen II	Intravenously	3 weeks, after last dose	Negative
5	11/21/24	Gradual Antigen II	Intravenously	1, 2, 4 weeks	Negative
7	11/21/24	Gradual Antigen II	Intravenously	2 weeks	Negative
8	10/30/24	Gradual Antigen II H	Intravenously	3 weeks	Negative
					Not tested with Antigen II H
9	10/30/24	Gradual Antigen II H	Intravenously	3 weeks	Negative
12	10/30/24	Gradual Antigen II H	Intravenously	3 weeks	Negative
10	10/30/24	2.5 Antigen II H	Intravenously	3 weeks	Negative
11	10/30/24	2.5 Antigen II H	Intravenously	3 weeks	Negative

Antigen II. For inoculation, Antigen I in a dilution of 1 to 5 was made with physiologic salt solution, Mandler filtered, and kept in refrigerator. Further dilution was made at time of using.

Antigen II H. Part of the Antigen II was held at 100° for one hour on water-bath, and stored in the refrigerator.

Treatment was of two sorts: gradual, in which the dose began with 50 pollen units and ended with 50,000, given intravenously at two to three day intervals; and massive, in a single dose of 125,000 units given intravenously. Both dosages were borne well, the animals gaining in weight.

At various intervals, complement-fixation tests were made using the standard technic of Kolmer for the Wassermann test, with inactivated serum in a constant amount of 0.1 c.c. Since it was impossible to titrate the antigen for its antigenic value, Antigen II was arbitrarily chosen, being neither hemolytic nor anticomplimentary in that amount. (In greater concentration there was a tendency to hemolyze cells, apparently because of the action of the glycerin.) The results are given in Table I. Out of 10 animals, tested with an antigen of about 1 per cent pollen content, only 2 showed complement fixation, and these not to a satisfactory degree. With the incidental use of more concentrated solutions of the pollen, a larger number of positive fixations were secured. These findings seemed to necessitate further experiments.

In the first place, pollen may be considered as weak in antigenic substances, thereby requiring the use of relatively large quantities for a measurable production of antibodies. Along with this the influence of extractives may be in the direction of altering the antigenic quality and of lessening the quantity of active substances. In view of these possibilities the use of untreated pollen in larger doses was undertaken.

In the second place, for the tests the question of a satisfactory antigen was to be determined. Theoretically, this should be made simply and of the lowest dilution not giving anticomplementary action. It would seem that a simple physiologic salt solution would occasion the least changes in the

TABLE II

ANTIGENS USED FOR COMPLEMENT-FIXATION TESTS

NO.	POLLEN	VARIATION IN PREPARATION	P_H AS USED	POLLEN % AS USED
1.	Artem. Calif.	Repeated aq. extraction and concentration by evaporation and in vacuum.		2
4.	" "	First residue treated with 9%, salt-filtrate brought to 0.9%.		2
8.	Artem. Trident.	Mill-ground, paper filtered.	7.2	2.5
9.	" Calif.	Mill-ground, paper filtered.	7.4	1
10.	" "	Mill-ground, paper filtered.	7.4	
12.	" "	Mill-ground, paper filtered.	7.2	2.5
13.	" "	Normal salt extraction.	8.2	
14.	" "	Mill-ground, adjusted at once to P_H 7.4, paper filtered.		2.5
15.	" "	Mill-ground, 10% normal salt, frozen 17 times in CO_2 snow and ether, adjusted to P_H 7.4.		1
16.	" "	Mill-ground.	7.4	0.5
17.	" "	Normal salt solution.	7.4	1
18.	" "	1% salt solution.	7.0	1
19.	" "	1.25% $NaHCO_3$ adjusted.	7.0	1
20.	" "	1% NaCl with equal parts glycerin, adjusted.	7.0	1
21.	" Dracone.	1% NaCl, adjusted.	7.0	1

TABLE III

COMPLEMENT-FIXATION IN SERA-RABBIT SERIES 200

DATE	ANTIGEN	VARIATION IN TECHNIC	RESULTS	PRECEDING TREATMENT
2/28/25	No. 1		Negative	3 weeks after first inoculation as above
			Negative	
			Negative	Normal control
3/ 2/25	No. 6	1½ doses complement. sensitized cells	Serum anticomlementary	Three inoculations
			Serum anticomlementary	Three inoculations
			xxxx	Two inoculations
3/ 3/25	No. 10 diluted x 2	Fixed 1 hour water-bath	anticomplementary	Normal
			xxxx	Three inoculations
			xxx	Three inoculations
			xx	Two inoculations (stored serum)
3/12/25	Nos. 12, 13, 14		xxx	Three inoculations
			anticomplementary	Three inoculations

natural qualities of the substances, and that the dilution to be used should be a matter of simple titration.

But in the course of making these adjustments, a third factor was forced into consideration; and it is here, possibly, that the source of the discrepancies in the various reports on pollen fixation may largely lie—that is, in the tendency of rabbit serum to fix complement nonspecifically. This was pointed out particularly by Kolmer,[1] working with lipoidal extracts where inactivated normal rabbit serum gave fixation in about 40 per cent, and with bacterial antigens where the percentage of fixation reached fifty-six, with the conclusion that "this property of rabbit serum of absorbing or fixing complement in a nonspecific manner should be emphasized and better known, for when this animal is used for the purpose of immunization with the object of subsequently conducting complement-fixation tests with the serum, the

TABLE IV

COMPLEMENT-FIXATION IN SERA-RABBIT SERIES 115
(HETEROLOGOUS INOCULATIONS)

DATE	ANTIGEN	VARIATION IN TECHNIC	RESULT	PRECEDING TREATMENT
3/ 2/25	6	1½ doses complement, sensitized cells	xxxx	One inoculation
			xxx	" "
3/ 3/25	10, diluted 1:1	1 hour water-bath fixation	xxxx	" "
			Negative	" "
			Negative	Normal serum
3/ 6/25	4.9		Anticomplementary	One inoculation
			x	" "
		Results practically the same with both antigens	xxxx	" "
			xxxx	" "
3/12/25	12, 13, 14	As above	Anticomplementary	" "
3/14/25	14	1½ doses complement in presence of known negative serum	xx	" "
			Negative	" "

factor of nonspecific complement fixation enters and may greatly modify the interpretation of results."

Since the chief concern was to secure evidences of antibody rather than to prove, at this point, the value of a given extractive method, the success of Parker[2] in securing precipitins by inoculating rabbits with whole pollen intraperitoneally prompted the use of this method.

Five light young rabbits, Series 200, were given 500 mg. of Artemisia californica ether-treated, to remove oils and to partially sterilize, suspended in salt solution, and given intraperitoneally. One animal reacted at once with a violent chill; another was found dead after three days. After three weeks the 4 were given 250 mg. each. Eight days later one was found dead of pneumonia, without signs of peritonitis. In the mesenteries were found numerous pollen masses of pea and millet sizes which, microscopically, were found to consist of amorphous material and many intact pollen granules. This method would then correspond because of the slow absorption to that of the graduated dosage type. A third rabbit was very emaciated after two weeks and was bled to death. The remaining two survived a third dose of 250 mg. Another group of 5 grey rabbits, Series 115, bore without ill effects a treatment with 250 mg.; and 5 gained weight under the graduated intravenous dosage of 2 per cent extract of pollen in normal salt and sodium bicarbonate as used by Parker for precipitin tests. This extract was used also as antigen in the complement-fixation tests, which followed the standard technic of Kolmer, except that perforce, the titration for antigenic value was omitted. The antigens, freshly prepared, were passed through hard filter paper rather than through the Berkefeld filter in order to conserve the protein content. A practically clear solution resulted if the suspension was first centrifuged at high speed. The preparation of the various lots of antigen are listed in Table II.

Tables III, IV and V give the results of 36 tests of sera from treated animals. Seventeen were positive, 6 were negative or doubtful and 7 anti-complementary. But at the same time, 6 normal sera gave 2 positive and

TABLE V

COMPLEMENT FIXATION IN SERA-RABBIT SERIES 125

DATE	ANTIGEN	VARIATION IN TECHNIC	RESULTS	PRECEDING TREATMENT
3/ 6/25	4, 8, 9		xxxx	Seven doses
			xxxx	" "
			2 anticomplementary	" "
			4 | 8 | 9	" "
			0 | xxx | x	" "
			xx	Normal
3/12/25	12, 13, 14		Negative with 14	Seven doses
			Anticomplementary	" "
			12 | 13 | 14	" "
			xxx | xxxx | xx	
			xx | xxx | xx	Normal
3/14/25	14	1½ doses complement in presence of known negative serum	xxxx	"
			Negative	"

TABLE VI

COMPLEMENT FIXATION IN RABBIT SERUM*

NORMAL SERUM	ANTIGEN			NO ANTIGEN
	15	16	17	
0.12 c.c.	xxxx	xxx	xxxx	0
0.18 c.c.	xxxx	xx	xxx	0
0.24 c.c.	xx	x	xx	0
IMMUNE SERUM				
0.12 c.c.	xxxx	xxxx	xxxx	0
0.18 c.c.	xxx	xx	xxx	0
0.24 c.c.	xxx	0	0	0

*In each case 0.12 c.c. serum contains a double hemolytic unit, and the results are in degree of fixation.

1 anticomplementary reaction. These very irregular results, including fixation with a heterologous antigen, A. tridentata, suggested the use of a technic allowing the use of unheated serum, thereby reducing the tendency to nonspecific fixation,[3] and conserving the antibody.

In studying complement fixation in human sera, using streptococcus in suspension as antigen, Burbank and Hadjopoulos[4] secured satisfactory results with a system adapted to meet the difficulties inherent in the inadequacy of a weak antigen and in the deleterious effect of heat on antibody in process of inactivation.

The active serum was titrated for its complement content in the presence of 0.5 c.c. of 0.5 per cent sensitized sheep cells. The test set-up was two series of four tubes each, in which increasing complementary units of sera were pipetted. To one series was added 0.5 c.c. of the antigen of a dilution twice that of the anticomplementary unit, determined in the presence of pooled negative sera. As prepared, the antigens were not found to be hemolytic in double the dose used, either before or after adjustment; but in dilutions lower than the anticomplementary values, there was a tendency to decolorize without clearing—as with saponin—inhibited by adjustment toward a P_H of 7.0. After one hour's fixation in the water-bath, the sensitized sheep cells were added. A typical reaction is recorded in Table VI.

With a hemolytic system so adjusted that the unit of complement fell around 0.1 c.c. serum, six sera, read as positive under the Kolmer system, gave with antigens 15, 16, 17 no more fixation than the pooled sera of three normal rabbits, using the complement of each serum, determined before and during the test. The work was checked, with the same results, by using pooled sera of both immunized and normal animals to obviate the excessive manipulation incident to the self-complement titration, using antigens 18-21. From this one could only say that the antibody titer and the anticomplementary titer lay too close together to make demonstration of antibody content of value in this case.

DISCUSSION

Where the immunizing agent and the test antigen are used in states as little changed as possible and as nearly alike as possible, failure to secure satisfactory immune body reactions (errors of technic not considered) must be due either to the known poverty of pollen in antigenic substances, or to an actual deficiency in antibody content. On the other hand, where reactions are secured, they must be discounted by the tendency of rabbit serum to fix nonspecifically unless this condition is suitably controlled. From the experiments here reported, it would seem that both factors may be responsible for the variable results reported for complement fixation in pollen-treated animals and that the test is inadequate as a means of standardizing pollen extracts.

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THE ANTICOMPLEMENTARY REACTION OF BLOOD SERUM TO THE KOLMER COMPLEMENT-FIXATION TEST CONTROLLED BY THE REACTION TO THE KAHN PRECIPITATION TEST*

By S. William Becker,† M.D., Rochester, Minn.

A STUDY on the anticomplementary reaction to the Kolmer complement-fixation test on both blood serum and cerebrospinal fluid was presented by Sanford in May, 1925. All the samples of cerebrospinal fluid on which this reaction was obtained were from patients almost definitely proved to have syphilis, and 59 per cent of the samples of blood serum were from syphilitic patients. As a continuation of this study, I have performed the Kahn precipitation test on 112 samples of blood serum which have yielded anticomplementary reactions to the Kolmer test. These samples were obtained from sixty-nine patients seen at the Mayo Clinic.

Kolmer's standardized quantitative complement-fixation test was employed, except that only the first, second, and control tubes were used, and in each case the serum was inactivated for twenty minutes instead of fifteen. The degree of reaction in the three tubes was recorded in all but ten instances. In the latter the degree of reaction in only the control tube was known; this was designated as "strongly anticomplementary" in two instances and as "weakly anticomplementary" in eight instances. The Kahn precipitation tests were performed immediately after the Kolmer tests, about twenty-four hours after withdrawal of the blood. The serum had been left on the clot at room temperature. The method of Kahn was followed with a slight modification, the tubes being shaken vigorously by hand instead of being shaken by machine. For convenience the results 3+ and 4+ are designated "strongly positive," and the results 1+ and 2+ "weakly positive" (Tables I, II, III, IV, V, VI,

Table I

Strongly Positive Reaction to Kahn Test with Definite History or Signs of Syphilis

RESULTS OF KOLMER TESTS	CASES
444 on all examinations (three to six)	3
444 on one to three examinations, with additional positive reactions	10
44 in first two tubes and 1 to 4 in control tube on different examinations, with additional positive reactions in all but one instance	11
Less than 44 in first two tubes and 1 or 2 in control tube, with additional positive reactions	3

Table II

Strongly Positive Reaction to Kahn Test without Definite History or Signs of Syphilis

RESULTS OF KOLMER TESTS	CASES
444 on one examination (Case 1)	1
444 but negative on same serum twenty-four hours later (Case 2)	1
442 on one examination (Case 3)	1

*Submitted for publication, May 1, 1926.
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TABLE III

WEAKLY POSITIVE REACTION TO KAHN TEST WITH DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULT OF KOLMER TEST	CASE
444 on one examination, with additional positive reactions	1

TABLE IV

WEAKLY POSITIVE REACTION TO KAHN TEST WITHOUT DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULTS OF KOLMER TESTS	CASES
444 on four and two examinations, respectively, with additional negative reactions (Cases 4 and 5)	2
Slightly anticomplementary on one examination, with additional negative reactions (Cases 6 and 7)	2

TABLE V

NEGATIVE REACTION TO THE KAHN TEST WITH DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULTS OF KOLMER TESTS	CASES
Negative in first two tubes and 4 in control tube, with additional positive reactions	1
212 on one examination with positive cerebrospinal fluid	1

TABLE VI

NEGATIVE REACTION TO THE KAHN TEST WITHOUT DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULTS OF KOLMER TESTS	CASES
444 on all examinations (one to four)	11
444 accompanied by weakly anticomplementary, negative, or positive reactions	6
44 in first two tubes and less than 4 in control tube, with additional positive and negative reactions in one case	2
Less than 44 in first two tubes and 1 to 4 in control tube with additional strongly anticomplementary, weakly anticomplementary, positive or negative reactions	13

VII). No definite diagnosis could be made in the cases in Tables II and IV, and the case histories and clinical findings are, therefore, briefly presented. Some of the patients did not remain for complete investigation for syphilis.

CASE HISTORIES AND CLINICAL FINDINGS

CASE 1.—A man, aged forty-six, complained of backache and malaise of one year's duration. He was found to have infected teeth and tonsils and chronic infectious arthritis of the lumbar spine. There was no history or clinical signs of syphilis. The reaction to the Kolmer test on the blood was anticomplementary (444), and the reaction of the Kahn test was 4. The blood was withdrawn shortly after the ingestion of a heavy meal. No further investigation for syphilis was made.

CASE 2.—A woman, aged twenty-eight, complained of indigestion of twelve years' duration. Duodenal ulcer was diagnosed. There was no history or clinical signs of syphilis. The reaction to the Kolmer test on the blood was anticomplementary (444), but was negative on the same serum twenty-four hours later. The reaction of the Kahn test was 4. No further investigation for syphilis was made.

CASE 3.—A woman, aged sixty, was brought to the clinic by relatives. For fifteen months she had had delusions of persecution, based on auditory and visual hallucinations. Cooperation was so poor that a satisfactory examination could not be made. There was no history of syphilis. The reaction to the Kolmer test on the blood was anticomplementary (442) and that to the Kahn test 4.

TABLE VII

CLASSIFICATION OF THE SYPHILITIC CASES

TYPE	CASES
Congenital syphilis	1
"Latent" syphilis	1
Gumma of skin or mucous membrane	5
Leukoplakia of tongue	1
Gastric syphilis	1
Hepatic syphilis	2
Syphilitic aortitis	3
Undifferentiated neurosyphilis	5
General paresis	8
Tabes dorsalis without gastric crisis	1
Tabes dorsalis with gastric crisis	1

TABLE VIII

DIAGNOSIS OF THE NONSYPHILITIC CASES

DISEASES	CASES
INFECTIOUS	
Draining sinus of hand	1
Multiple foci of infection	2
Poliomyelitis (recent)	1
Scarlet fever (recent)	1
Vincent's angina	1
Chronic infectious arthritis	5
Dental and tonsillar sepsis	1
Tonsillar sepsis	1
Chronic cholecystitis	2
Chronic nephritis	2
Renal calculus	1
Prostatitis	1
Chronic yellow atrophy of liver with "infected ascites"	1
NONINFECTIOUS	
Epilepsy	1
Arteriosclerosis of central nervous system	1
Dermatitis venenata	1
Erythema multiforme	1
Acrodermatitis chronica atrophicans	1
Ophthalmoplegia, unexplained	1
Carcinoma of tongue	1

CASE 4.—A man, aged forty-three, complained of stomach trouble of five years' duration. Diagnosis was made of cholecystitis, septic tonsils, and alcoholic gastritis. There was no history or clinical signs of syphilis. The Kolmer test on the blood was anticomplementary (444) on four occasions, in spite of the fact that the patient drank alcohol shortly before each withdrawal of blood. Craig and Nichols have shown that the ingestion of alcohol tends to cause negative Wassermann reactions. The reaction to the Kahn test was negative on the first two samples of serum, and weakly positive on the last two. Examination of the spinal fluid was negative. He was advised to return for reexamination.

CASE 5.—A woman, aged thirty-three, complained of swelling in the thyroid region, of fifteen years' duration. Diagnosis was made of colloid goiter with adenomas. There was no history of syphilis. The pupils were irregular, unequal, and did not react to stimulation by light. The reaction to the Kolmer test on the blood was anticomplementary (444) on two occasions. The reaction to the Kahn test was weakly positive on the first sample of serum and negative on the second. No further investigation for syphilis was made.

CASE 6.—A man, aged forty, complained of hemorrhage from the lungs of one and one-half years' duration. Diagnosis of bronchiectasis was made. His wife had syphilis. Five years previously he had had a negative reaction to a provocative test.[5] The Kolmer test on the blood was weakly anticomplementary and the Kahn test weakly positive. His spinal fluid had never been examined.

CASE 7.—A woman, aged forty-four, complained of "liver trouble" of six months' duration. At operation she was found to have chronic cholecystitis and biliary cirrhosis. There was no history of syphilis. The reaction to the Kolmer test on the blood was negative. The Kahn test was not performed on this serum. On account of enlargement of the left lobe of the liver, the patient was given a provocative injection of arsphenamine. The results of the five daily Kolmer tests were: negative, weakly anticomplementary, and negative three times. The reaction to the Kahn tests were: weakly positive twice and negative three times. The spinal fluid was normal. The husband's reactions to the Kolmer and Kahn tests were negative, and his cerebrospinal fluid was normal. The diagnosis was indeterminate as regards syphilis.

Many of the patients with positive Kolmer reactions, never stronger than 33, were investigated by the multiple-procedure diagnostic attack of Stokes, but in no instance could definite signs of syphilis be elicited. The difficulty in evaluating the weakly positive and moderately positive Kolmer reaction in the absence of history and evidence of syphilis will be discussed elsewhere.[1]

Six of the patients with general paresis (Table VII) and the tabetic patient with gastric crisis had just completed a course of malarial treatment. Each patient had had positive Kolmer reactions previously, but never an anticomplementary reaction. The absence of primary and secondary syphilis from the list may be of significance, or it may be attributable to the paucity from the list may be of significance (Table VIII).

COMMENT

If the cases in Table II are included in the syphilitic, and those in Table IV in the nonsyphilitic cases, there are thirty-three (48 per cent) syphilitic and thirty-six (52 per cent) nonsyphilitic cases. If the cases in which "artificial" anticomplementary reactions were obtained on the serum (the cases in which malarial treatment was being given for neurosyphilis) are disregarded there are twenty-six (42 per cent) syphilitic and thirty-six (58 per cent) nonsyphilitic cases. This percentage is somewhat less than that of the syphilitic cases in Sanford's series (59 per cent), but both series are too small to permit of final conclusions.

There was one instance of anticomplementary reaction on both blood and cerebrospinal fluid, a combination not present in Sanford's series.

Three-quarters of the nonsyphilitic patients were suffering from infectious disease. This, coupled with the fact that anticomplementary reactions were produced in a certain percentage of neurosyphilitic patients by inoculation with malaria, suggests infection as a cause for the anticomplementary reaction.

My results are in accord with those of Kolmer, who says, "If the serum is very slightly anticomplementary and the front tube shows complete inhibition of hemolysis, the reaction is in all probability positive. If the rear tube, however, shows marked inhibition of hemolysis, indicating that it is highly anticomplementary, the result cannot be determined, but a retest with fresh serum must be made."

SUMMARY

Of a series of sixty-nine patients whose blood serums were anticomplementary on 112 occasions, 48 per cent were found to be syphilitic. More than three-fourths of the nonsyphilitic patients were suffering from infectious disease. There is no evidence that the substance which fixes complement without antigen also produces a positive precipitation reaction. Avoidance of the anticomplementary Kolmer reactions by substituting the Kahn test does not permit of satisfactory evaluation in all cases. However, the Kahn precipitation test is a valuable addition to the multiple-procedure diagnostic attack of Stokes in the study of patients whose serum gives an anticomplementary reaction by the Kolmer complement-fixation test.

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COMPARISON BETWEEN THE KAHN FLOCCULATION TEST, THE KOLMER-WASSERMANN TEST, AND THE RUEDIGER-WASSERMANN TEST*

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IN A previous report I[1] showed that my modification of the Wassermann test is more sensitive than the Kolmer modification of the Wassermann test. During the last year the Kahn flocculation test has attracted a great deal of attention and many reports are appearing in the medical journals. I shall briefly refer to some of these reports. Levin[2] made parallel Kahn and Wassermann tests on 2,542 serums with 92.2 per cent agreement in the results. Boas[3] reports 91 per cent agreement with the Kahn and Wassermann tests. Owen and Cope[4] prefer the Wassermann test to the Kahn test. Redfield[5] prefers the Kahn test because of its simplicity. Houghton[6] came to the conclusion that the Kahn test possesses superior qualities over the Wassermann test and other precipitation tests now in use, because it is much more simple and less time-consuming. The United States Navy[7] adopted the Kahn test as the official serologic test for syphilis and yaws. In a later report Owen and Cope[8] show 93.8 per cent agreement with the Kahn test and the Kolmer-Wassermann test. Occasionally they had a positive result with the Kolmer-Wassermann test and a negative result with the Kahn test and a

*From the Hollywood Clinical Laboratory, Hollywood, California.
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TABLE I

COMPARISON OF THE KAHN FLOCCULATION TEST, THE KOLMER-WASSERMANN TEST, AND THE RUEDIGER-WASSERMANN TEST

NO. OF SPECIMEN	KAHN TEST	KOLMER-WASSERMANN	RUEDIGER-WASSERMANN Units per c.c.	REMARKS
1	Negative	Negative	20	Syphilitic history
2	+++	44400	250	Syphilis
3	+++	44400	200	Syphilis
4	++++	44400	200	Syphilis
5	++++	44420	350	Syphilis
6	±	40000	36	Treated syphilis
7	Negative	Negative	20	Husband syphilitic
8	+	20000	36	Syphilis
9	++	43000	50	Syphilis
10	+++	44300	180	Syphilis
11	+	40000	100	Syphilis
12	++++	44430	500	Syphilis
13	Negative	Negative	6	Treated syphilis
14	+	21000	50	Syphilis
15	+++	44420	300	Syphilis
16	+++	44300	150	Husband of No. 10
17	Negative	Negative	40	Treated child of No. 7
18	++++	44400	350	Syphilis
19	Negative	Negative	20	Treated syphilis
20	Negative	Negative	6	Early chancre
21	++++	44410	200	No. 20, 11 days later
22	+++	44300	150	Syphilis
23	++++	44420	400	Syphilis
24	++++	44444	1500	Secondary syphilis
25	++++	44410	500	Syphilis
26	+	20000	36	Syphilis
27	++++	44400	300	Syphilis
28	Negative	Negative	15	Treated syphilis
29	+++	44400	250	Syphilis
30	+	33200	200	Treated syphilis
31	Negative	Negative	100	Treated syphilis
32	++	44400	200	No. 21, a month later
33	++++	44400	150	Syphilis
34	++	44000	75	Syphilis
35	++++	44420	400	Syphilis
36	++	40000	60	Syphilis
37	Negative	Negative	12	Treated syphilis
38	Negative	Negative	20	Treated syphilis
39	+++	44000	100	Syphilis
40	Negative	Negative	20	Treated syphilis
41	++	44000	36	Treated syphilis
42	+	10000	20	Treated syphilis
43	+++	44400	100	Syphilis
44	++	44200	36	Syphilis
45	Negative	Negative	12	Syphilis, stomach trouble
46	+++	44430	50	Secondary syphilis
47	Negative	Negative	12	Treated syphilis
48	++++	44420	100	Syphilis
49	Negative	Negative	6	Wife of No. 50
50	+++	44400	100	Secondary syphilis
51	+++	44440	300	Syphilis
52	Negative	44000	50	Treated syphilis
53	+	44000	36	Syphilis
54	Negative	Negative	12	Treated syphilis
55	+	44000	36	Syphilis
56	+++	44400	60	Chancre 3 weeks old
57	Negative	Negative	20	Treated syphilis
58	+++	44400	60	No. 56, 3 days later
59	++	34300	30	Treated syphilis
60	+++	44400	70	Syphilis

TABLE I—CONT'D

NO. OF SPECIMEN	KAHN TEST	KOLMER-WASSERMANN	RUEDIGER-WASSERMANN Units per c.c.	REMARKS
61	+	Negative	15	No. 49, 17 days later
62	+++	44400	70	Syphilis
63	+	11000	24	Treated syphilis
64	Negative	Negative	12	Treated syphilis
65	+++	44420	200	Chancre
66	++++	44430	300	Wife of No. 65
67	Negative	31000	36	Treated syphilis
68	Negative	32000	36	Chancre 3 (?) days old
69	Negative	Negative	12	Chancre 6 days old
70	Negative	Negative	20	No. 69, 2 days later
71	Negative	Negative	4	Spinal fluid, syphilis
72	++	44400	50	No. 70, 5 days later
73	+++	44400	150	Syphilis
74	Negative	Negative	12	Treated syphilis
75	Negative	Negative	12	Treated syphilis
76	++	44100	36	Treated syphilis
77	+	44200	36	Syphilis
78	++++	44443	350	Syphilis
79	+++	44410	200	Syphilis
80	+++	44430	200	Syphilis
81	+++	44400	50	Syphilis
82	++++	44440	400	Syphilis
83	+	44400	70	Syphilis
84	++++	44441	400	Syphilis
85	+	22000	36	Syphilis
86	+	11000	6	Spinal fluid, syphilis
87	+	01000	6	Spinal fluid, syphilis
88	+	32000	10	Syphilis
89	+	Negative	36	Syphilis
90	+++	44400	200	Syphilis
91	+++	44400	200	Syphilis
92	Negative	Negative	4	Syphilis
93	++	12000	36	Treated syphilis
94	Negative	23200	36	Treated syphilis
95	Negative	Negative	12	Treated syphilis
96	+	Negative	12	Treated syphilis
97	++++	44440	300	Syphilis
98	Negative	44200	24	Treated syphilis
99	+	22000	6	Treated syphilis
100	Negative	Negative	12	Treated syphilis

few cases gave positive results with the Kahn test and negative results with the Kolmer-Wassermann test. Giordano[9] made parallel Kahn and Kolmer-Wassermann tests on 2,540 serums and the results agreed in 96 per cent of the cases. Occasionally one test gave a positive result and the other test gave a negative result. In a clinical study of 110 cases Kelly[10] ran the Kahn flocculation test and the Kolmer-Wassermann test on the same serums. In 95.45 per cent of the tests the results agreed. The serums of three persons who were known to be syphilitic gave positive results with the Kahn test and negative results with the Kolmer-Wassermann test and the serum of one person gave a negative result with the Kahn test and a positive result with the Kolmer-Wassermann test.

METHODS

The Kahn test was done as described by Kahn and with antigen kindly supplied by Kahn. In the Kolmer-Wassermann test I used the human hemolytic system, otherwise I followed Kolmer's directions. The Ruediger-Was-

sermann test was done in accordance with the descriptions previously given,[1, 11, 12, 13] except for the following modification. To 100 c.c. of the antigen 50 mg. of cholesterol was added, the complement was always diluted 1:10, and 1.5 unit of hemolytic amboceptor was used.

My present report deals with the results I obtained with the Kahn flocculation test, the Kolmer-Wassermann test, and my own modification of the Wassermann test on 260 consecutive specimens. Of the 260 specimens, 160 gave negative results with all three methods and 100 specimens gave positive results with one or more methods. Among those that gave negative results by all three methods were at least two bloods that came from persons who clinically were syphilitic. One patient had two almost typical Charcot joints and the other was diagnosed as syphilitic marasmus with arteriosclerosis. Both patients were given antisyphilitic treatment and marked improvement followed. The spinal fluids were not examined.

The more important part of this report deals with the specimens that gave positive results, and are given in detail in Table I.

The accompanying table shows the results that were obtained with the serums that were positive by one or more methods. Those which gave positive results need no further discussion but some explanation is given on those serums in which the results disagree.

Serum No. 1 came from a young married woman in her first pregnancy who was admitted to a hospital for some minor trouble. I was studying the Wassermann test in pregnancy at the time, and therefore included her. The Kahn test gave a negative result, the Kolmer-Wassermann test gave a negative result and my method showed 20 fixing units per cubic centimeter of serum. There were no other signs of syphilis, the positive Ruediger-Wassermann test was ignored and the patient was sent home. About six weeks later her family physician sent me a specimen of her blood for Wassermann test and advised me that she had a miscarriage and that he obtained a syphilitic history. The serum was retested and the results were identical with the first.

Serum No. 7 was obtained from a married woman who has two living syphilitic children, and a tabetic husband, and has had several miscarriages. Repeated Wassermann tests done at other laboratories gave negative results, while I got negative results with the Kahn and Kolmer tests and 20 units fixation with my method. In early primary syphilis my method always gave positive results before positive results were obtained with the Kahn test or with the Kolmer test as is shown by Serums Nos. 20, 49, 68, 69 and 70. Under treatment the Kahn test and the Kolmer test became negative much sooner than my method; Serums Nos. 13, 17, 19, 28, 31, 37, 38, 40, 45, 47, 52, 54, 57, 64, 67, 74, 75, 89, 92, 94, 95, 96, 98 and 100 show these results. With spinal fluid from syphilities my method gave positive results when the Kahn test and the Kolmer test gave negative results as is shown by No. 71. Serum No. 45 came from a patient who had severe stomach trouble. There was a history of syphilis contracted more than twenty years before. The Kahn test and the Kolmer test gave negative results and with my method I demonstrated 12 fixing units per cubic centimeter of serum. The patient was given

antisyphilitic treatment and he improved rapidly. In a previous attack of apparently the same nature a gastroenterostomy was done, probably because the Wassermann test gave a negative result.

SUMMARY

Parallel Kahn flocculation tests, Kolmer-Wassermann tests and Ruediger-Wassermann tests were done on 260 consecutive specimens.

Of these 260 specimens 160 gave negative results by all three methods and 100 gave positive results by one or more methods. Among the 160 specimens that gave negative results by all three methods were at least two which came from patients who were clinically considered chronic syphilities. They were treated as syphilities and improved greatly. The blood serum only was tested.

All of the 100 specimens that gave positive results by one or more methods came from syphilities and all gave positive results by the Ruediger-Wassermann test, 70 gave positive results by the Kahn test, and 72 gave positive results by the Kolmer test. With three specimens the Kahn test gave positive results while the Kolmer test gave negative results, and with five other specimens the Kolmer test gave positive results and the Kahn test gave negative results. There was 70 per cent agreement between the Kahn flocculation test and the Ruediger-Wassermann test and 72 per cent agreement between the Kolmer-Wassermann test and the Ruediger-Wassermann test. Of the specimens that gave positive results by the Ruediger-Wassermann test and negative results by the Kahn flocculation test and the Kolmer-Wassermann test, four were blood serums from patients with primary syphilis, two were blood serums from more or less chronic syphilities that had always given negative results with the Wassermann test, one was a spinal fluid from a syphilitic, and the others were blood serums from treated syphilities.

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1680 North Vine Street.

THE RÔLE OF THE WASHINGS OF STREPTOCOCCUS SCARLATINAE IN SCARLET FEVER*

By N. S. Ferry, Ph.B., M.D., and L. W. Fisher, B.S., Detroit, Mich.

FOR carrying out the cutaneous reaction, referred to as the Dick reaction, for determining susceptibility to the Streptococcus scarlatinae and, presumably to scarlet fever, and also for prophylactic inoculation against the disease, the Dick toxin, as originally described by its sponsors[1] was prepared by passing the water of condensation of a sheep's blood agar growth of the scarlet fever streptococcus through a Berkefeld V filter. The filtered culture containing the soluble toxin from the organisms growing in the condensation fluid was diluted according to its requirements.

Later the Dicks, and also Zingher,[2] after the method of Williams, Hussey, and Banzhaf,[3] in New York, used a toxin prepared from a broth culture—the Dicks by the addition of sheep blood to the media, while Zingher advocated the addition of horse blood for enrichment purposes—the cultures being incubated, at the usual temperature, from three to six days. The toxin has also been obtained in plain broth by Kirkbride and Wheeler[4] and in 0.2 per cent glucose broth by ourselves—in fact it has been described by several investigators as having been obtained in almost any medium in which the Streptococcus scarlatinae will develop. The toxin, therefore, while not noticeably toxic to animals in the strengths obtained at present, has been described as an extracellular toxin from its action on the human.

The authors, following a method first reported by them in June, 1924,[5] whereby the washings of various organisms were successfully used as antigens for immunizing purpose, prepared an antigen in like manner from the washings of the Streptococcus scarlatinae, which not only produced the cutaneous susceptibility reaction in susceptible individuals but stimulated an active immunity against the toxin as well. The results with these washings of the Streptococcus scarlatinae corresponded very favorably with those produced with scarlet fever toxin, prepared in the usual manner from broth cultures, obtained from the Hygienic Laboratory, Washington. This shows that the same antigenic substance was present in both the washings and the toxin.

The work on the washings of the Streptococcus scarlatinae was reported[6] at the meeting of the Association of Immunologists in Washington, April, 1924, as follows: "Recently with washings prepared from a streptococcus isolated from scarlet fever we have been able to produce a positive skin reaction in individuals susceptible to scarlet fever, which reaction would become negative if the washings were mixed with convalescent scarlet fever serum, similar to the results obtained by others with the Dick toxin." A detailed account of this experimental work is given at this time.

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When this type of antigen is prepared from bacterial washings the organisms are incubated for twenty-four hours on solid media and washed off with salt solution, either with or without preservative. The suspension of organisms, which usually contains an unappreciable amount of water of condensation, is mechanically shaken for a few minutes and immediately passed through a Sharples centrifuge, leaving a clear watery solution containing a very small percentage of bacterial proteins. To this type of antigen the name ectoantigen was given, as it was clearly not of the extracellular type, nor was it of the endocellular type, and to distinguish antigens prepared in this way from others and for clinical purposes, the name immunogen was used.

As washings from such organisms as the pneumococcus, typhoid bacillus, pertussis bacillus, gonococcus, and streptococci, other than the Streptococcus scarlatinae, gave evidence of antigenic properties of extremely high immunizing value, it was thought probable that the washings of the scarlet fever streptococcus would behave in a similar manner. With that thought in mind preliminary tests were carried out on members of our laboratory staff giving negative histories to scarlet fever, with favorable results.

Later tests were carried on at three other institutions in Detroit: The Protestant Orphan Asylum, St. Vincent's Orphan Asylum, and St. Francis Home for Orphan Boys.

At the first institution all of the children had previously been given the cutaneous test with the regular toxin, and the susceptible ones given three immunizing doses of the toxin. Retests of these positive cases two months after the last immunizing with the antigen prepared by washing the Streptococcus scarlatinae, controlled by the Government standard toxin, showed that 80 per cent had remained successfully immunized for two months.

At St. Vincent's Orphan Asylum an epidemic of scarlet fever was in progress at the time the work was instituted, so that only those were tested who were considered normal and were not convalescing from the disease. Of these there were 195 and all were skin-tested with the washings on one arm and controlled with the standard toxin on the other. Of the 44 giving positive reactions half were immunized with three doses of the regular toxin and half with three doses of the washings, and all were retested two months after with the washings controlled as previously with the standard toxin. The first dose of the toxin and also of the washings contained an equivalent of 250 skin-test doses of toxin per cubic centimeter, the second dose contained 500 skin-test doses, and the third, 1,000 skin-test doses. All injections were given at intervals of one week. While rather disagreeable general reactions were recorded in three cases of the older girls following the injections of the toxin and also several severely swollen arms were noted, none of these symptoms were evidenced in the cases where the washings were used. On retest two months after the last immunizing dose 85 per cent of the cases gave negative reactions to the intracutaneous injection of the material, showing that 15 per cent were still susceptible to the disease, if the test is as reliable as it is thought to be.

It is interesting to note that this work was undertaken during an epidemic of scarlet fever at St. Vincent's Asylum, and not another case was

reported after the prophylactic injections were started. Whether the prophylactic injections had anything to do with the abating of the epidemic, we are not able to state. It would seem plausible, however, and because of the fact that half of the remaining children were immunized with toxin prepared in the regular way and half with the washings, it is only fair to assume that each product had an equal share in the successful termination of the epidemic.

At the St. Francis Home for Boys, all of the boys, 530 in number, were given the cutaneous test with washings, controlled with the standard toxin, and 92 were found to be positive reactors. These were all immunized with the washings, a dosage being employed much weaker than that used in the previous experiment at St. Vincent's Asylum, and about half the total number of skin tests being used. Even with this much smaller amount of antigen being used, it was found that 57.7 per cent of the susceptibles were successfully immunized; they were retested two months after the last immunizing dose.

Since this work with washings of the Streptococcus scarlatinae was first reported, it has been corroborated by Henry and Lewis,[7] in so far as the preparation of the antigen is concerned, in a report to the Medical Research Council of England. They used a similar method of preparation for their toxin, and then further precipitated the antigen with various volumes of alcohol and desiccated it. Favorable results were reported by them with dried material diluted to original volume with salt solution, when this material was used for the skin test. Prophylactic inoculations were not undertaken by these authors.

DISCUSSION

That the Streptococcus scarlatinae produces a soluble extracellular antigen, toxic in nature for the human, there is no doubt. Ample proof to substantiate this is at hand, especially in the production of the rash after large doses of this antigen given subcutaneously and, also, in the production of an antiserum, with this antigen which will neutralize scarlet fever toxin.

That this antigen is also of the nature of an ectoantigen has been shown by the above experiments with the washings.

It is very evident, therefore, that this antigen differs in many respects from that produced by the diphtheria and tetanus bacilli, as it has never been shown that washings from twenty-four-hour growths of these organisms on solid media produce antigens very toxic in nature, or that the toxin from the Streptococcus scarlatinae is very toxic for animals even in large doses.

CONCLUSIONS

Washings of the Streptococcus scarlatinae grown on solid media contain an antigen which, when injected intracutaneously into the human, will produce the cutaneous susceptibility test called the Dick test, similar to the broth filtrate.

These washings will also produce a prophylactic immunity against the scarlet fever toxin similar to that produced by the toxin itself.

The specific antigen of the streptococcus seems to be both extracellular and ectoantigenic in nature.

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Specialist in Pathology Needed at Knoxville, Iowa, Hospital

The United States Civil Service Commission states that there is a vacancy in a position of specialist in pathology at the Veterans' Bureau Hospital at Knoxville, Iowa, and that applications are being received for the position.

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LABORATORY METHODS

A COMPARISON OF THE FOLIN-WU AND NEW BENEDICT METHODS FOR THE ESTIMATION OF BLOOD SUGAR

By Arnold E. Osterberg, Ph.D., and Joy Strunk, B.A., Rochester, Minn.*

THE Folin-Wu procedure for the estimation of blood sugar has been adopted as a routine method in many laboratories. As a result physicians have come to regard 100 mg. of glucose for each 100 c.c. of blood, in a laboratory report, as approximately the normal value.

It is not theoretically correct to assume that all of the reduction obtained when the Folin-Wu copper reagent is added to a protein-free blood filtrate is due to glucose, since it is well known that when this reagent is applied to normal urine, values are obtained for the glucose present which are two or three times that known to be correct. In spite of this, however, the method has served admirably for the routine procedure in the clinical laboratory.

Since the recent publication by Benedict of a modified Folin-Wu copper reagent it was interesting to determine whether it was worth while, from a clinical standpoint, to alter the normal values for blood sugar and to change the procedure for the estimation of blood sugar from that of Folin-Wu to Benedict's newer procedure.† Benedict says that his new solution when applied directly to urine gives only one-tenth of the error of the Folin-Wu procedure, and that the results obtained are comparable when applied to urine that has been treated with Lloyd's reagent. The principal difference which Benedict has initiated in his modified solution is that the copper reagent has been varied so that the amount of citrate in the original solution has been increased, whereas the concentration of copper and carbonate has been decreased. Also, a small amount of sodium bisulphite has been added to increase the amount of cuprous oxide produced by a given amount of sugar. For the development of the color of the cuprous oxide, Benedict's uric acid reagent is used, to which has been added 5 per cent formaldehyde to prevent any increase of color by the sodium bisulphite. By this method Benedict has obtained as normal values for blood sugar, an average of 75 mg. for each 100 c.c. of blood, and he believes that even this figure may be too high. He would place the normal value at approximately 60 mg. for each 100 c.c. of blood. Folin's figures were not so low with the new method. In fact, he says that if care is taken to insure the use of fresh solutions and to prevent the oxidation of the sodium bisulphite by atmospheric oxygen, results are

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†Benedict has in a more recent paper again changed the method. This article, however, concerns itself only with the modifications first described.

obtained which are practically the same as those given by the Folin-Wu procedure.

In order to compare the two methods we determined simultaneously the glucose content of four blood filtrates by the two procedures. Pure glucose was then added to the filtrate in varying amounts and the total glucose content redetermined (Table I).

In general the error of recovery by Benedict's new method is less than by the Folin-Wu procedure. It would seem from these data that the amount of reduction obtained from a given amount of glucose is more nearly quantitative by the Benedict procedure. None of the errors by the Benedict procedure are of particular clinical significance since the greater ones are present only in those filtrates with high sugar values.

As a comparison of the two procedures in the routine estimation of blood sugar for clinical purposes, we have taken as a basis for our opinion glucose estimations made simultaneously on the same blood filtrates by the two methods. The results were obtained by the analysis of 194 consecutive blood specimens taken in the laboratory, with no regard to clinical diagnosis (Table II).

TABLE I

COMPARISON OF RECOVERY OF GLUCOSE ADDED TO BLOOD FILTRATES

SAMPLE	FILTRATE AND GLUCOSE, MG. FOR EACH 100 C.C.	MG. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD		CALCULATED NUMBER OF MG. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD		ERROR IN MG. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD	
		FOLIN-WU	BENEDICT	FOLIN-WU	BENEDICT	FOLIN-WU	BENEDICT
A		93	80				
A	20	112	95	113	100	− 1	− 5
A	60	162	136	153	140	+ 9	− 4
A	100	206	184	193	180	+13	+ 4
A	140	250	224	233	220	+17	+ 4
A	180	302	266	273	260	+29	+ 6
A	220	352	306	313	300	+39	+ 6
A	260	408	356	353	340	+55	+16
B		93	80				
B	20	113	100	113	100	0	0
B	60	162	138	153	140	+ 9	− 2
B	100	188	178	193	180	− 5	− 2
B	140	230	224	233	220	− 3	+ 4
B	180	276	258	273	260	+ 3	− 2
B	220	326	300	313	300	+13	0
B	260	362	332	353	340	+ 9	− 8
C		95	83				
C	20	119	103	115	103	+ 4	0
C	60	158	150	155	143	+ 3	+ 7
C	100	198	184	195	183	+ 3	+ 1
C	140	246	216	235	223	+11	− 7
C	180	302	266	275	263	+26	+ 3
C	220	346	310	315	303	+31	+ 7
C	260	396	350	355	343	+41	+ 7
D		97	90				
D	20	121	108	117	110	+ 4	− 2
D	60	166	153	157	150	+ 9	+ 3
D	100	192	198	197	190	− 5	+ 8
D	140	242	238	237	230	+ 5	+ 8
D	180	284	278	277	270	+ 7	+ 8
D	220	324	322	317	310	+ 7	+12
D	260	380	380	357	350	+23	+30

TABLE II

THE GLUCOSE CONTENT OF BLOOD ESTIMATED BY THE FOLIN-WU AND BENEDICT'S NEW PROCEDURE

NUMBER	GM. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD			NUMBER	GM. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD		
	FOLIN-WU	BENEDICT	DIFFERENCE		FOLIN-WU	BENEDICT	DIFFERENCE
1	.073	.069	.004	61	.100	.098	.002
2	.083	.077	.006	62	.101	.090	.011
3	.083	.075	.008	63	.101	.089	.012
4	.084	.076	.008	64	.101	.086	.015
5	.085	.078	.007	65	.101	.086	.015
6	.085	.068	.017	66	.101	.085	.016
7	.088	.082	.006	67	.101	.082	.019
8	.088	.080	.008	68	.102	.090	.012
9	.088	.078	.010	69	.102	.083	.019
10	.089	.078	.011	70	.103	.089	.014
11	.089	.075	.014	71	.103	.078	.025
12	.090	.077	.013	72	.105	.096	.009
13	.090	.078	.012	73	.105	.095	.010
14	.091	.087	.004	74	.105	.093	.012
15	.093	.089	.004	75	.105	.092	.013
16	.093	.081	.012	76	.105	.089	.016
17	.093	.081	.012	77	.105	.087	.018
18	.093	.082	.011	78	.105	.087	.018
19	.094	.082	.012	79	.105	.086	.019
20	.094	.080	.014	80	.105	.083	.022
21	.095	.086	.009	81	.106	.087	.019
22	.095	.084	.011	82	.107	.093	.014
23	.095	.085	.010	83	.107	.091	.016
24	.095	.082	.013	84	.107	.084	.023
25	.095	.083	.012	85	.107	.090	.017
26	.095	.080	.015	86	.108	.088	.020
27	.095	.080	.015	87	.108	.093	.015
28	.095	.075	.020	88	.108	.095	.013
29	.095	.085	.010	89	.108	.085	.023
30	.096	.087	.009	90	.109	.091	.018
31	.096	.080	.016	91	.110	.097	.013
32	.096	.079	.017	92	.110	.095	.015
33	.096	.084	.012	93	.110	.095	.015
34	.097	.090	.007	94	.110	.089	.021
35	.097	.091	.006	95	.111	.106	.005
36	.097	.087	.010	96	.111	.100	.011
37	.097	.083	.014	97	.111	.097	.014
38	.097	.084	.013	98	.111	.088	.023
39	.097	.081	.016	99	.111	.085	.026
40	.098	.094	.004	100	.113	.103	.010
41	.098	.087	.011	101	.113	.098	.015
42	.098	.085	.013	102	.113	.100	.013
43	.098	.085	.013	103	.113	.099	.014
44	.100	.092	.008	104	.114	.100	.014
45	.100	.090	.010	105	.114	.099	.015
46	.100	.090	.010	106	.115	.091	.024
47	.100	.090	.010	107	.117	.108	.009
48	.100	.090	.010	108	.118	.100	.018
49	.100	.089	.011	109	.118	.097	.021
50	.100	.089	.011	110	.119	.100	.019
51	.100	.088	.012	111	.119	.108	.011
52	.100	.087	.013	112	.121	.098	.023
53	.100	.087	.013	113	.121	.089	.032
54	.100	.087	.013	114	.121	.105	.016
55	.100	.087	.013	115	.121	.115	.006
56	.100	.085	.015	116	.121	.111	.010
57	.100	.085	.015	117	.121	.111	.010
58	.100	.084	.016	118	.121	.108	.013
59	.100	.083	.017	119	.122	.110	.012
60	.100	.075	.025	120	.122	.100	.022

TABLE II—CONT'D

NUMBER	GM. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD			NUMBER	GM. OF GLUCOSE FOR EACH 100 C.C. OF BLOOD		
	FOLIN-WU	BENEDICT	DIFFERENCE		FOLIN-WU	BENEDICT	DIFFERENCE
121	.123	.109	.014	158	.198	.170	.028
122	.125	.108	.017	159	.204	.182	.022
123	.125	.078	.047	160	.206	.184	.022
124	.125	.105	.020	161	.208	.196	.012
125	.125	.105	.020	162	.210	.194	.016
126	.125	.098	.027	163	.212	.210	.002
127	.125	.086	.039	164	.214	.196	.018
128	.127	.108	.019	165	.214	.196	.018
129	.128	.121	.007	166	.214	.190	.024
130	.129	.117	.012	167	.216	.210	.006
131	.131	.111	.020	168	.216	.206	.010
132	.136	.125	.011	169	.216	.193	.023
133	.138	.132	.006	170	.222	.208	.014
134	.145	.136	.009	171	.222	.216	.006
135	.146	.138	.008	172	.230	.214	.016
136	.148	.134	.014	173	.230	.210	.020
137	.150	.148	.002	174	.238	.218	.020
138	.150	.129	.021	175	.238	.182	.056
139	.152	.148	.004	176	.240	.212	.028
140	.153	.153	.000	177	.242	.234	.008
141	.153	.138	.015	178	.242	.232	.010
142	.153	.132	.021	179	.250	.238	.012
143	.153	.137	.016	180	.250	.242	.008
144	.156	.126	.030	181	.254	.250	.004
145	.157	.129	.028	182	.258	.238	.020
146	.160	.130	.030	183	.258	.226	.032
147	.160	.157	.003	184	.266	.264	.002
148	.160	.152	.008	185	.266	.222	.044
149	.164	.154	.010	186	.296	.294	.002
150	.168	.160	.008	187	.302	.296	.006
151	.170	.166	.004	188	.302	.274	.028
152	.172	.168	.004	189	.332	.332	.000
153	.178	.168	.010	190	.352	.320	.032
154	.182	.160	.022	191	.360	.340	.020
155	.182	.174	.008	192	.376	.350	.026
156	.184	.180	.004	193	.400	.392	.008
157	.186	.184	.002	194	.412	.400	.012

Thus it may be seen that Benedict's new procedure invariably yields lower results than the Folin-Wu procedure. The differences, however, are not as great as those reported by Benedict, the average difference for the total series being 14.5 mg. per cent.

In the ninety cases in which the values determined by the Folin-Wu method are below 0.110 gm. for each 100 c.c. of blood, and may be considered as normal or subnormal values, the average difference is 13 mg. per cent.

There are eighteen samples with sugar values determined by the Folin-Wu method of 0.100 gm. for each 100 c.c. of blood. The Benedict method gave results averaging 12.4 mg. per cent lower, although in one case the difference was only 2 mg. per cent and one sample differed 25 mg. per cent.

CONCLUSIONS

1. The modified copper solution of Folin-Wu as reported by Benedict yields results considerably lower than by the original Folin-Wu procedure. The differences are not consistent in our hands, but on the average are approximately 12 mg. per cent lower in a series of 194 consecutive samples.

2. The recovery of glucose added to blood filtrates is more satisfactory by the Benedict modification.

3. Routine procedures will be continued on the second modification of Benedict.

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VOLUME MEASUREMENT OF BLOOD PLATELETS*

By C. M. Van Allen, M.D., Iowa City, Iowa

BLOOD platelet estimation by volume is a departure from current practice, but the following method is presented because of its relative simplicity and reliability, and because of the significance of platelet volume in itself.

PRINCIPLE

If a specimen of blood, freshly drawn and mixed with an anticoagulant, be allowed to stand, the corpuscles will sink gradually and leave a supernatant fluid of whitish, ground-glass appearance in which platelets remain uniformly suspended for hours. As is well known, the platelet content of this fluid is the same per unit volume as was that of the whole specimen before sedimentation, and this fact has been made use of in the platelet counting method of Thomsen[1] and modifications by Gram,[2] Schenk and Spitz,[3] and Reimann.[4] In the present procedure, instead of counting the platelets in the supernatant fluid from such a preparation, they are extracted from it by centrifugalization and their volume is measured.

APPARATUS

Instruments† required are the "thrombocytocrit," sedimentation chamber, 10 c.c. Record syringe, and a centrifuge capable of 3500 r.p.m. The thrombocytocrit (Fig. D) is a glass capillary tube with overlying chamber of 6 c.c. capacity. The capillary bore is of 0.03 c.c. capacity, and the tube is graduated in a finely divided scale. Its lower end may be closed by applying the accompanying spring sealing clip[5] (Fig. C) in the manner illustrated (Fig. B). The sedimentation chamber (Fig. A) is a spherical flask of 20 c.c. capacity.

*From the Laboratories of the Rockefeller Institute for Medical Research, New York City, and the University Surgical Clinic, Munich, Germany.

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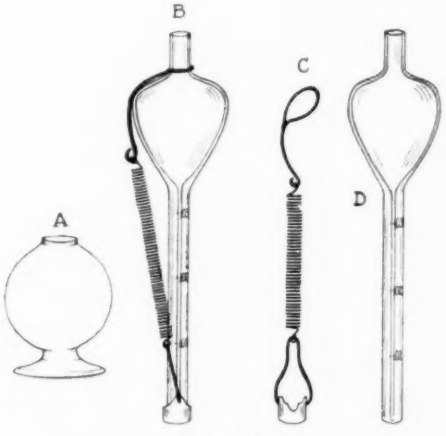
†The instruments may be obtained from Arthur H. Thomas Co., Philadelphia and Firma Arno Haak, Jena, Germany.

METHOD

Six c.c. of a 1.3 per cent solution (isotonic[6]) of sodium oxalate is drawn into the syringe, the air-bubble being excluded, and 4 c.c. of blood is added by venipuncture. This is deposited in the sedimentation chamber and more solution added, to 20 c.c. The chamber contents are then mixed thoroughly with the syringe, and are set aside for three and one-half hours for sedimentation. By means of syringe and needle 5 c.c. of the supernatant fluid is carefully removed from the chamber, including a little of the corpuscular sediment, enough to give to the fluid a faintly reddish tint. This is then deposited in the thrombocytocrit, the sealing clip having been already applied, and the instrument is centrifugalized at about 3500 r.p.m. for one and one-half hours (see below). On examination, the capillary of the thrombocytocrit will be found partly filled with sediment arranged in two sharply defined strata, below, a column of red substance representing the corpuscles included in the



Figs. A-D.

fluid, and, above this, an ivory white column composed of platelets. The extent of the white column is then read from the scale, indicated in parts of a cubic centimeter. By shifting the decimal point in the figure obtained two places to the right, the result will indicate the percentage of platelets by volume in whole blood.

RESULTS

Data obtained from normal individuals of three species are given in the table. Experimental work in which the method has been used is being reported elsewhere.

TECHNICAL PRECAUTIONS

The blood must be taken directly and rapidly from the circulation and its immediate and thorough mixture with the anticoagulant assured, in order to prevent the platelet lysis which starts in blood directly after shedding. Smaller amounts of blood than that specified may be employed if desired and the readings correspondingly adjusted. Sedimentation should not be contin-

ued longer than four hours. A small amount of corpuscles is included with the fluid for centrifugalization, in order that the bottom of the capillary may be filled with this sediment (red) and the stratum of platelets elevated to a position where its limits can easily be read. After adding the fluid, the thrombocytocrit should be centrifugalized as soon as possible. Counterbalancing must be accurately attended to as regards both the amount and the distribution of weight because of the unusual shape of the instrument. As counterweight a second thrombocytocrit filled with water serves most efficiently. The duration of centrifugalization necessary to procure complete packing of the platelets in the capillary bore depends upon the particular centrifuge used (radius of rotation), and is to be determined, to begin with, by reading the

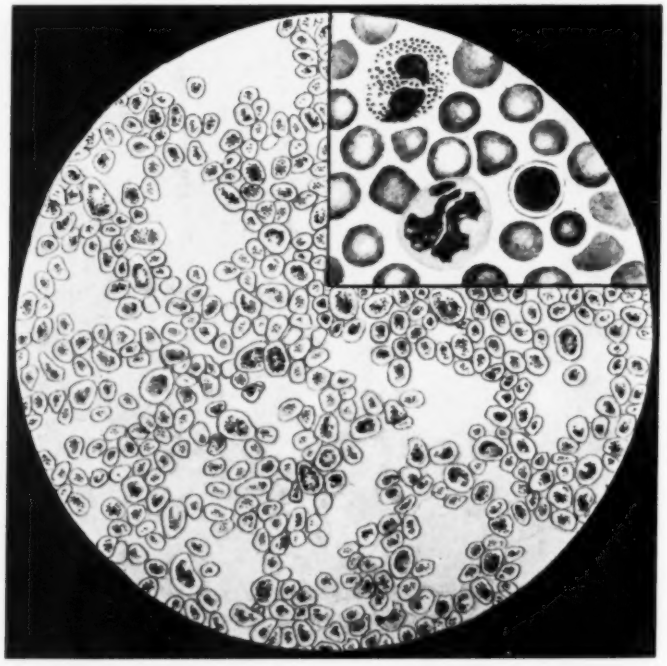


Fig. E.—Drawing of platelet substance (high oil magnification) as removed from thrombocytocrit and stained. Insert: for comparison, blood corpuscles at same magnification.

same specimen repeatedly, i.e., after one hour's centrifugalization and at fifteen-minute intervals thereafter. A typical result is: 0.80 per cent, 0.75 per cent, 0.73 per cent, 0.72 per cent, 0.72 per cent, 0.71 per cent, 0.71 per cent, 0.71 per cent; here a period of one and three-fourths hours has reduced the reading to 0.72 per cent, which is close enough to its final value, and this amount of centrifugalization may be adopted for routine use. Particular care must be given in cleaning the capillary of the thrombocytocrit, for platelets tend to adhere to the wall of an unclean tube and form a thin coating on the glass above the white column, which, if extensive, vitiates the reading. Before each use the instrument should be immersed in cleaning fluid (sulphuric acid-sodium dichromate solution) for six hours or more, then rinsed carefully and dried. The oxalate solution should be clear.

TABLE I

SPECIES	NUMBER EXAMINED	NORMAL PLATELET VOLUME (PER CENT OF WHOLE BLOOD)	
		RANGE	AVERAGE
Man	15	0.35–0.67	0.49
Dog	15	0.70–1.50	1.04
Rabbit	15	0.40–0.72	0.53

COMMENT

The nature of the white substance which is measured in the thrombocytocrit, when removed from the capillary and stained, is shown in the drawing (Fig. E). For comparison, the insert shows blood corpuscles of the same magnification. White blood cells are to be found among the platelets but are so rare as to have no volumetric significance, even under the circumstances of leucocytosis. The homogeneous consistency of the platelet substance is also demonstrated by the fact that it fails totally to appear in the capillary, when test is made of the blood of an animal treated effectually with a thrombocytolytic agent. The considerable individual variation here reported in normal blood platelet volume is in agreement with that known in normal counts of these structures. Platelet volume measurements run parallel, for the most part, with values obtained by counting, but the parallelism is not complete, nor is that to be expected, since the size of the platelet may vary considerably in disease[7] and between species. Measurement of the volume relations of the platelets in the blood would seem to be of distinct advantage in estimating thrombocytic function, since, apparently, platelets act quantitatively in the blood coagulative process by virtue of their chemical composition rather than as individual elements, and assist also in the control of bleeding (thrombosis) by mass accumulation.[8] The method is entirely mechanical, microscopy and the personal element therein being eliminated. It permits the saving of considerable time, where a number of tests are required, and sedimentation of several specimens is done simultaneously. The thrombocytocrit may be used, also, to measure the amount of any finely divided material in fluid suspension, as the bacterial content of vaccines, or for the purpose of obtaining blood platelets in pure deposit for study.

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A NOTE ON THE KOCH AND McMEEKIN METHOD FOR THE DETERMINATION OF NITROGEN; WITH SPECIAL REFERENCE TO THE NONPROTEIN NITROGEN OF BLOOD AND URINE*

By H. A. Davenport, M.D., St. Louis, Missouri

THE direct nesslerization, or micro-Kjeldahl method for the determination of nitrogen described by Koch and McMeekin employs 30 per cent hydrogen peroxide (Merck's superoxol or Kahlbaum's perhydrol) to assist in the oxidation of carbonaceous material formed in the process of digestion with sulphuric acid. Since 3 per cent hydrogen peroxide (U. S. P.) is more readily available than the 30 per cent peroxide, and may be handled with impunity, it seemed desirable to determine whether it might be satisfactorily substituted in the determination of nonprotein nitrogen of blood and urine.

Most 3 per cent peroxides contain one-fifth grain of acetanilid per fluid ounce, so experiments were made to determine the extent of error caused by this substance. One-tenth of a gram of acetanilid was added to 1 c.c. of half-concentrated sulphuric acid and digested as in nonprotein nitrogen determinations. When diluted and nesslerized, no color was produced. If, however, acetanilid free peroxide was added to such a digesting mixture, nitrogen was liberated as ammonia. Duplicate 5 c.c. portions of four different brands of U. S. P. peroxide were then digested, diluted, and nesslerized, and read against a standard of equal volume which contained 0.25 mg. of nitrogen. A value of 0.046 mg. per c.c. was obtained for three of the samples, while the fourth (Parke, Davis and Co.) gave too little color to be read. No acetanilid content was declared on the label of the latter. Since the theoretical amount of nitrogen available from peroxide with the usual acetanilid content is approximately 0.042 mg. per c.c., it appears to be liberated quantitatively. Therefore, a correction of 0.002 mg. N per drop (20 to 25 drops to the c.c.) of peroxide used will bring the accuracy of results within the limit of error of the colormetric method.

A series of experiments on blood filtrates, on urine, and on a standard urea solution gave satisfactory results when 5, 10, and 15 drops of peroxide were used, provided the above correction were applied. Three drops were often sufficient to clear blood filtrate.

The peroxide should be dropped directly into the hot acid mixture, and not allowed to run down the side of the tube. If it is added in this manner immediately or within five to ten seconds after removal of the flame, a minimum amount is required.

*From the Department of Biochemistry, Washington University Medical School.
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Example of the application of the correction for U. S. P. peroxide (containing acetanilid):

1. 5 c.c. blood filtrate digested with 1 c.c. half concentrated H_2SO_4 and addition of 5 drops nitrogen free 3 per cent peroxide — N found — 0.160 mg.
   equivalent to N per 100 c.c. of blood — 32.000 mg.
2. Same as above except that 5 drops of U. S. P. peroxide were used— N found — 0.176 mg.
   Less correction of 0.002 mg. per drop, 0.010 = 0.166 mg.
   or N per 100 c.c. blood — 33.000

SUMMARY

Ordinary 3 per cent hydrogen peroxide (U. S. P.) instead of the 30 per cent solution may be used to clear the sulphuric acid digests of blood and urine in micro nonprotein nitrogen determinations by the Koch-McMeekin procedure, provided a correction be made for nitrogen derived from the acetanilid. With this slight change, this method is in our hands preferable in that the nesslerized solutions are always clear and the separation of silica, which often occurs with the phosphoric acid mixture, is avoided.

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A SIMPLER METHOD FOR THE PREPARATION OF POTASSIUM PYROGALLATE SOLUTION FOR METABOLIC RATE DETERMINATIONS*

By Francis F. Schwentker, B.S., Schenectady, N. Y.

MOST basal metabolic rate determinations include in their technic the analysis of expired gases. For this purpose Haldane gas analysis tubes are generally used, in which the oxygen content of the gas is adsorbed by a potassium pyrogallate solution. Definite directions for the preparation of this solution are give by Haldane[1] and the finished product adsorbs the oxygen faultlessly, but its preparation is difficult and uncertain. At times it seems impossible to obtain the intermediate potassium hydroxide solution with a density of 1.55 at room temperature, and often, the finished product having been obtained, it persists in crystallizing instead of remaining a deep wine color.

Following are directions for the preparation of this potassium pyrogallate solution which eliminate much of the difficulty usually experienced and have the decided advantage of always producing the required solution. In addition, the finished product is identical with that called for by Haldane.

*From the Laboratory of the Ellis Hospital, Schenectady, N. Y.
Received for publication, August 20, 1926.

To 600 gm. of stick potassium hydroxide (not purified by alcohol),* 300 c.c. of distilled water are added and the hydroxide dissolved by placing the mixture on a cold water-bath and heating the bath to boiling. As soon as complete solution has been effected, the volume is roughly measured.

The density of this hot solution is then determined by quickly weighing 100 c.c. in a volumetric flask and should be 1.517 at the temperature the solution will assume if weighed sometime within three minutes after removal from the water-bath. If it has not this density, the solution must be poured back with the remainder and after replacing on the water-bath, potassium hydroxide or water added according to whether the density is less or greater than 1.517.

When the correct density has been attained, the hot solution is poured into a glass stoppered bottle (with greased stopper) containing 9.7 gm. of Merck's pyrogallic acid for each 100 c.c. of the potassium hydroxide. The resulting mixture will be the required brown-green potassium pyrogallate solution which, after cooling, gradually assumes a deep wine color. It should be at least a month old before using, although, as Boothby and Sandiford[2] point out, the aging can be hastened by exposing to the air for a few minutes.

It will be found that if the above method is followed the density of the potassium hydroxide solution at the first determination is usually somewhat higher than 1.517. The amount of water necessary to dilute to the proper density can be quickly and easily approximated by the following formula:

$$\text{No. c.c. diluting water necessary per 100 c.c. solution} = \frac{100\ (\text{Density}) - 151.7}{51.7}$$

The density of the solution will usually be found correct after the addition of the volume of water computed by the above formula, but the actual density should be more accurately determined by weighing.

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*The potassium hydroxide, purified, sticks, of Merck's are, according to the manufacturer, not purified by alcohol and are suitable for use in this preparation.

NEW APPARATUS FOR MEASURING THE SPONTANEOUS MOTILITY OF ANIMALS*

By Curt P. Richter, Ph.D., and Ging H. Wang, Ph.D., Baltimore, Md.

DESCRIPTIONS have been given in previous papers of cages used in investigating the different factors involved in the production and modification of spontaneous activity of animals (rats, etc.), (Richter,[1] 1922, 1926[2]), (Wang, 1923, 1925[3, 4]). Because these cages had a number of serious defects which curtailed their usefulness for this type of work, we have designed a new cage very much simpler in both construction and operation.

A photograph of three racks of these cages is shown in Fig. 1. The racks, seventy-two inches long, seventy-six and one-quarter inches high, and fourteen and one-half inches wide, are made of three-quarter inch angle iron with a one-eighth inch galvanized iron partition. Each one contains sixteen cages arranged in four tiers. The cage, attached to the partition, consists of a living compartment with a food box and a watering tube, and a revolving drum with a device for registering the revolutions. The living cage communicates with the revolving drum through a circular hole three inches in diameter in the partition. The dimensions of the individual cages and of the surrounding framework are shown in Fig. 2.

The living compartment is built entirely of one-half inch mesh wire-cloth, with the side toward the partition open. In order to divert practically all of the animal's activity to the drum where it can be measured, this living cage is made as small as possible—eleven inches long, five inches high, and three inches wide. It can be easily removed from the two hooks fastened to the partition to support it. The small recess built in one end to hold the food cup is covered with a special arrangement to prevent the spilling of food, and is so constructed that the cup can be removed from the outside without disturbing the animal. An inverted watering tube, graduated in cubic centimeters (Richter, 1926), is fastened to the cage with a phosphorus bronze clip at the end opposite to the food box.

The revolving drum is made of one-eighth inch mesh wire-cloth, six inches wide, mounted on a wooden disc thirteen inches in diameter and one-half inch thick. The disc is attached to the axle of a ball-bearing bicycle hub and the hub is firmly bolted to the partition. A small hole in the partition accommodates the other end of the axle.

The revolutions of the drum are registered automatically by means of a device shown in Fig. 2. A small brass arm one-half inch in length is fastened to that end of the axle which protrudes through the hole in the partition. A joint connection is made between this arm and the lever of a cyclometer by means of an aluminum rod. Thus, when the drum revolves,

*From the Psycho-Biological Laboratory, Johns Hopkins Hospital, Baltimore, Md.
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the aluminum rod fastened excentrically to the axle of the drum causes the lever of the cyclometer to be raised and lowered. In this way each revolution of the drum, clockwise or counter-clockwise, is registered. The cyclometer is attached to the partition in such a way that it can be read with little effort, and the whole arrangement is very simple in construction and operation. It is a modification of the method used by Stewart[7] (1898).

The racks are made very firm so that one animal may not stimulate another through vibrations set up by the revolving drum. Thus far we have found no indication of such interstimulation.

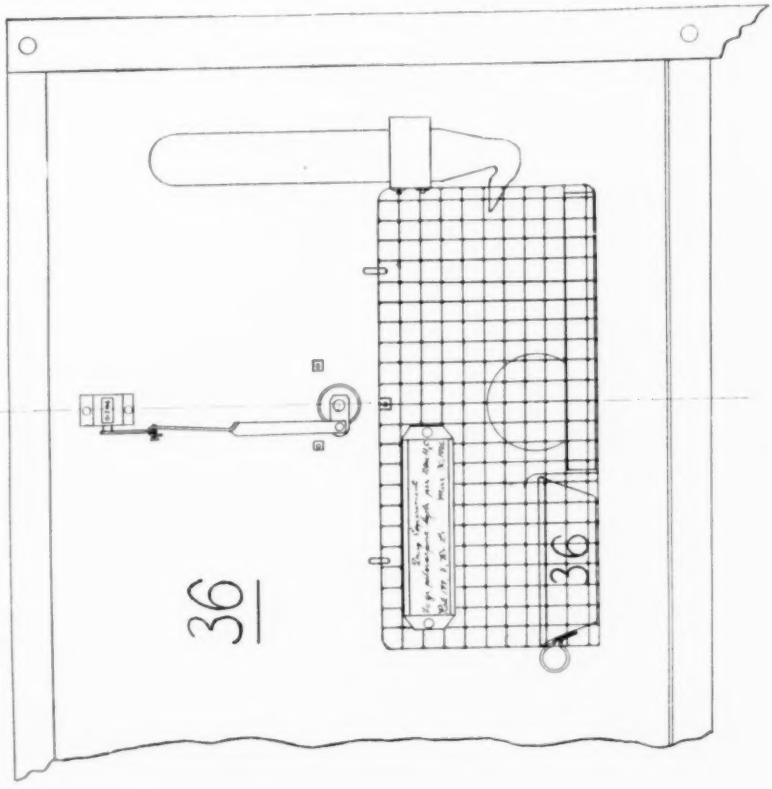
In designing these cages and stands we made a special effort to insure

Fig. 1.

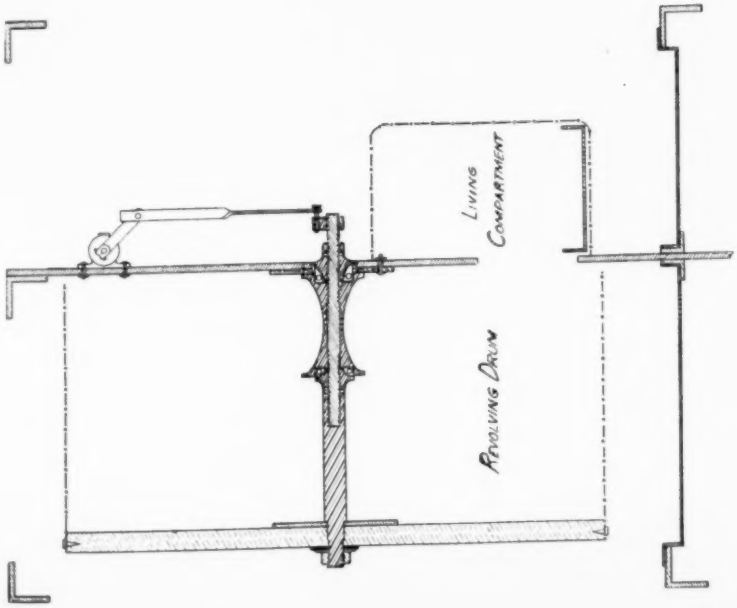
that they might be easily and thoroughly cleaned. Practically all parts of the cages can be detached, and the stands themselves are almost entirely free from cracks and crevices in which dirt and vermin can collect. Pans filled with sawdust are provided beneath the living cages and the drums to catch the urine and feces. By changing the sawdust in the pans frequently almost all of the unpleasant odor usually present in animal rooms can be eliminated.*

This arrangement of cages and method of recording activity have the following advantages. (1) The cages are very compact so that a large num-

*The cages were made after a design by E. A. Kalstner, Baltimore. The watering tubes were made by Levitt and Ferguson Co., Baltimore.



PLAN VIEW



CROSS SECTION

SCALE:

Fig. 2.

ber can be kept in a relatively small space. (2) They can be cleaned easily and frequently with slight disturbance. (3) The registering device is very simple and cannot be easily thrown out of adjustment. (4) The elimination of activity outside of the drum makes certain that most all of the animal's activity is registered. That this method is successful is shown by the fact that the animal often runs as much as ten to fifteen miles in twenty-four hours, and occasionally as much as twenty-seven miles.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Martin, C. J., and Lepper, E. H.: A Micro-method for the Estimation of the Hydrogen-Ion Concentration of Capillary Blood. Biochem. Jour., 1926, xx, 37.

Requirements.—1. A freshly prepared saline solution containing 0.7 per cent NaCl and 0.2 per cent potassium oxalate or 0.2 per cent sodium fluoride. Shortly before use, one volume of 0.02 per cent phenol red is added to 10 volumes of saline and the P_H adjusted to about 7.5 with N/50 NaOH containing the same amount of indicator. It is kept in a hard glass bottle protected from CO_2.

2. A set of standard tubes made of resistance glass, 7 cm. long and 2.5 mm. internal bore. These are filled with M/15 phosphate solutions of P_H varying from 7.3 to 7.6 at intervals of 0.05, to which one-tenth volume of 0.02 per cent phenol red has been added, and sealed off. These standards must be made up frequently as they fade.

3. A number of pieces of resistance glass tubing about 8 cm. long and of 2.5 mm. internal bore drawn out at each end to capillary dimensions so as to leave about 7.5 cm. from shoulder to shoulder. The glass tubing must be selected and prepared as described below under "special precautions."

Procedure.—A mark is made on one of the pieces of resistance tube at 0.5 cm. from the shoulder of one end, *A*. The tube is then filled from end *B* up to this mark with the saline containing phenol red and placed ready in a horizontal position. A large drop of blood is obtained from the finger or ear by a deep puncture and the end *B* of the tube, already filled with saline, is placed in it. End *A* is slightly depressed and the blood is allowed to flow in, pushing the saline in front of it, until the saline reaches *the beginning* of the capillary at end *A*. When this has occurred, end *B* is wiped dry and end *A* sealed in a peep-flame. As this end cools, blood is sucked into end *B* which is also sealed either in the flame or with sealing-wax. The blood and saline are at once mixed by rotating the tube between the fingers to avoid clotting. The corpuscles are then separated by spinning in a hand centrifuge for a couple of minutes and the color of the mixture of saline and plasma compared with that of the phosphate standards.

The matching is done by holding the tubes in a good light at an angle of 45° to a piece of white paper lying on the bench. The tubes should be held by the capillary ends so that they are not warmed by contact with the hand. Greater accuracy is obtained if they are placed in test tubes of water at 18° containing a slip of white filter paper as background.

If the unknown is found to lie between say 7.35 and 7.4 another standard of P_H 7.375 may be made up and the relation of the unknown to this determined. With a little practice, and P_H around 7.4, it is possible to place the unknown between two standards 0.025 P_H apart. The range of P_H over which such fine adjustment can be made will depend on the color vision of the individual worker. For most purposes, however, intervals of 0.05 in the P_H of the phosphate standards are close enough. This enables a determination to be made within $\pm$ 0.02 P_H.

Special Precautions.—1. The stock phosphate solutions must be made up with the greatest accuracy attainable. They must be kept in hard glass bottles and protected from evaporations and from CO_2 when withdrawing some by a trap of soda-lime on the inlet tube.

2. The indicator should only be added to the saline solution shortly before use and the P_H adjusted to 7.5.

3. The whole reliability of the method depends on the small glass tubes not furnishing alkali. Only resistance glasses are suitable and these must be individually tested as described below, as glass from the same batch varies.

Pieces of tubing are washed and left standing in boiled distilled water containing phenol red. If after a few hours the contents of the tubing become pinker the glass is unserviceable. If no change in color occurs, the tubing is dried, cut into lengths and the ends drawn out.

As most resistance glass yields alkali temporarily after being melted in the flame, the tubes are rinsed in distilled water and left to soak for some hours in boiled out distilled water containing phenol red. If no pink color develops inside the tubes they are fit for use. They are rinsed with distilled water and dried with alcohol. *They must not be dried in a hot oven.*

4. In filling the tubes the minimum of air necessary for easy sealing should be left and entrance of CO_2 from the peep-flame during sealing avoided.

5. Unless NaF is used, separation of the corpuscles and comparison with the standards should be undertaken forthwith. With oxalate, the P_H gradually falls from glycolysis. At room temperature no change can be detected after an hour, but at 38° a fall is discernible after a quarter of an hour. The use of fluoride as anticoagulant inhibits glycolysis (Evans). If 0.2 per cent NaF is employed the P_H generally remains constant for twelve hours at 18°. This is a convenience in clinical work, as the sample of blood can be taken and mixed with the saline at the bedside and the remaining stages proceeded with at leisure.

6. The colorimetric observations must be made at or about 18°. The P_H attributed to a particular color of the indicator in Sorensen's various phosphate mixtures is only valid for 18° so that if the comparison is made at another temperature the result will be incorrect. When using phenol red the error from this cause is of no great moment provided the temperature change does not exceed ± 3°, as this would lead to a difference of only 0.015 in P_H. Greater accuracy is obtained if the standard tubes and the blood-containing tubes are placed in test tubes of water at 18° as described above.

If Cullen's correction of −0.2 is applied to the P_H of human blood determined colorimetrically at 18-20° the result will closely approximate the P_H of arterial blood at body temperature.

Fowweather, F. S.: Determination of the Amount and Composition of Fat of Feces. I. Investigation of a "Wet" Method and Comparison with the "Dry" Method. Brit. Jour. Exper. Path., February, 1926, vii, 7.

A comparison of Saxon's method for the fresh specimen with the method described by the dry method of Cammidge.

The technic of the "wet" method is as follows:

The sample is first thoroughly mixed, using a pestle and mortar if necessary to ensure uniformity. If liquid, a portion of the stool weighing about 5 gm. is poured into each of three stoppered weighing bottles, and the weight of each portion obtained. One weighing bottle is placed in a steam oven and dried to constant weight in order to determine the percentage of dry matter contained in the stool. Each of the remaining portions is washed into a 100 c.c. graduated glass-stoppered cylinder of uniform width throughout. To the contents of one cylinder 3 c.c. of concentrated hydrochloric acid are added, and then distilled water to 30 c.c. The contents of the second cylinder are diluted to 30 c.c. also without the addition of any acid. If the stool is solid a quantity of about 10 gm. is placed in a weighed porcelain evaporating dish or crucible provided with a glass rod 2 to 3 inches in length, flattened at one end. Having obtained the combined weight of dish (and rod) and feces, a quantity of the latter weighing 2 to 3 gm. is withdrawn on the flattened end of the rod and introduced into the cylinder by touching the cylinder wall with it below the ground portion at the mouth. The dish and contents together with the rod, with any feces still adhering to it, are then reweighed. A second weighed quantity of feces is similarly transferred to the second cylinder, after which the dish, rod and contents are dried to constant weight in the steam oven. Acid and distilled water are added to the cylinders as in the case of liquid stools. To each cylinder are now added 20 c.c. of ether, and the cylinders vigorously shaken for five minutes. Then, after standing for a few moments, 20 c.c. of 95 per cent alcohol are added to the neutral cylinder and 17 c.c. to the acid cylinder. The alcohol is mixed with

the other contents by giving the cylinders a sharp circular movement. Some rise of temperature occurs, and the cylinders are therefore stood in cold water in the sink for a short time, after which the contents are vigorously shaken for five minutes. The cylinders are then allowed to stand to allow the ether layer to separate. If separation does not occur readily gentle movement of the cylinder in a circular direction often helps, with the previous addition of a few drops of alcohol in most cases or ether in others. Experience will indicate the best means to adopt in any particular case, but once this has been obtained satisfactory separation need cause no difficulty.

The upper layer is blown off into a fat extraction flask by converting the cylinder into a wash-bottle by the addition of a cork carrying glass tubes. The exit tube is turned up at the lower end to avoid any disturbance of the separating surface by upward currents in the liquid. The removal of the ether layer is made complete by addition to and removal from the cylinder of three successive 5 c.c. quantities of ether.

A second extraction is then made by adding another 20 c.c. of ether to each cylinder, shaking for five minutes, separating and transferring the ether layer as before. From the combined extracts in the flask the solvent is evaporated, and the residue is dried and dissolved in petroleum ether. The solution is filtered into a weighed flask and the petroleum ether evaporated off. After drying again the weight of extracted fat is obtained. Titration of the fat of the neutral extraction in benzene solution by N/10 sodium alcoholate completes the analysis. All results are calculated as a percentage of the dry matter of the feces. They are thus strictly comparable with the results obtained by the dry method.

The wet method is regarded as more accurate and less likely to lead to erroneous clinical deductions.

Wile, U. J., and Belote, G. H.: Syphilitic Alopecia, Its Relation to Neurosyphilis. Arch. Dermat. and Syph., April, 1926, xiii, 495.

Syphilitic alopecia of the essential type has a high associated incidence of meningeal syphilis, as indicated by spinal fluid findings.

The absence of the accepted criteria in the spinal fluid cannot, moreover, be accepted as absolute evidence of the absence of such involvement.

Microscopic study shows that the essential syphilitic alopecia is not due to any local pathologic disturbance of the scalp, or more specifically, of the follicular apparatus. It is therefore not a true syphilid.

Clinical analogy affords the suggestion that it is due to endocrine dysfunction as a result of association and involvement of the autonomic nervous system.

Symptomatic alopecia representing a smaller group of the entire syndrome is a true syphilid, apparently caused by a perifollicular plasmoma.

Jones, H. N., and Wise, L. E.: Cellobiose as an Aid in the Differentiation of Members of the Colon-Aerogenes Group of Bacteria. Jour. Bact., May, 1926, xi, No. 5, 359.

Cellobiose is a carbohydrate prepared from cellobiose octa-acetate by acetolysis, the method of preparation in detail to be published.

When added to broth in 0.5 per cent concentration it serves as a ready and accurate means of differentiation between E. coli and A. aerogenes, the latter forming gas and acid, the former producing neither.

Thompson, L.: The Blood Agar Plate for Spore-Forming Anaerobes. Jour. Bact., May, 1926, xi, No. 5, 305.

Attention is called to the availability of the blood agar plate for the isolation and grouping of spore-bearing anaerobes, some being hemolytic, some producing methemoglobin, and others being without effect on red blood cells.

The media used was Huntoon's hormone agar with 1.5 per cent agar, tubed in amounts of 10 to 12 c.c. Plates were poured after serial inoculation and incubated in a Novy jar exhausted by a combination of hydrogen and alkaline pyrogallol methods. When hydrogen

alone was used the procedure was called the "single method"; both methods combined were called the "double method."

The paper is illustrated with twenty-three microphotographs and presents a tabular, tentative classification of anaerobes on the basis of their characteristics when grown on blood agar plates under anaerobic conditions.

Greenbaum, S. S.: Error of Basing Serum Diagnosis of Syphilis on the Kahn Reaction Alone. Jour. Am. Med. Assn., April 24, 1926, lxxxvi, 1273.

Using both the Kolmer quantitative complement-fixation test and the Kahn floccula tion test as a routine, Greenbaum emphasizes that from three to four per cent of discrepancies occur. Either test may give occasional false negative reactions.

Occasionally, in treated cases, the Kahn test remains positive when the Kolmer test has become negative. The significance of these reactions has yet to be determined, although in a number of such instances the spinal fluid gave a positive Kolmer reaction.

Greenbaum believes that a dangerous error may be introduced if the Kahn test is relied upon as the sole means of serodiagnosis and that both tests should be used routinely.

Murphy, J. B.: Observations on the Etiology of Tumors. Jour. Am. Med. Assn., April 24, 1926, lxxxvi, 1270.

Using the same chicken tumor as was used by Gye, his experiments were repeated using in detail the methods described by him but also using control cultures of normal tissues.

Anaerobic "cultures" of chick embryo and rat placenta proved just as effective as so-called cultures of malignant tumors in activating chloroform treated filtrates of chicken sarcoma.

The necessity of assuming a cultivated living organism in the interpretation of Gye's results is eliminated.

Mills, H. R.: Comparison of the Kolmer Quantitative Test with the Routine Wassermann. Jour. Florida Med. Assn., February, 1926.

A series of 1328 tests is reported the results of which corroborate the delicacy and specificity of the reactions with this technic previously reported by numerous observers.

Sauthgate, H. W., and Carter, G.: Excretion of Alcohol in the Urine as a Guide to Alcoholic Intoxication. Brit. Med. Jour., March 13, 1926, 463.

Within the range of forensic medicine there is no subject upon which medical evidence is more unsatisfactory than that pertaining to drunkenness.

Of late years this diagnosis has become of great moment in connection with motor accidents and, in the absence of incontrovertible evidence, is a matter of great difficulty.

The authors present experimental evidence indicating that:

1. The alcohol in the blood is related to the amount of alcohol consumed when it is imbibed under constant conditions.

2. The relation of alcohol in the blood to alcohol in the urine is a fairly constant one in many circumstances.

3. The concentration of alcohol in the blood is related to the symptoms of intoxication of the central nervous system.

From the experimental evidence demonstrating these propositions a practical method was devised for the calculation of the blood alcohol concentration at the time of arrest by deducing the blood concentration from the urine alcohol concentration.

As the collection of specimens of urine from a prisoner suspected of drunkenness may involve some difficulty a special cell is used the discharge pipe of the urinal of which passes to another cell and empties into a receptacle. Nothing is said to the prisoner and the bladder is emptied voluntarily and without suggestion.

The time of arrest is noted and also the time of the collection of the specimen which is examined at once by the method described below.

If the figure 1.35 be taken as the ratio of urine alcohol to blood alcohol the latter can be calculated. Knowing this figure and the time interval between the arrest and the passage of the specimen, the blood alcohol at the time of arrest can be calculated from the fact that the alcohol concentration in the blood falls at the rate of about 12 mg. per hour per 100 grams of blood.

It remains to be determined by experiment, however, what blood alcohol concentration figure can be taken as the upper limit as regards the fitness of an individual to drive an automobile.

By the method described, however, the fact that an individual is "under the influence of liquor" may be rather definitely determined.

"The method of estimating alcohol in the urine is that of Cannon and Sulzer" (Cannon, R. K., and Sulzer, R.: Heart, London, April 5, 1924, xi, 148).

Reference to this paper gives a method for the estimation of alcohol in blood said to be applicable to other biologic fluids and which, presumably, is the method used by Sauthgate and Carter. The latter authors state that normal constituents of the urine do not interfere with the reaction and the presence of acetone bodies introduces only a minimal error, 8 mg. of acetone being equivalent to 1 mg. of alcohol under experimental conditions. A routine test with Fehling's solution acts as a guard on this.

The method described by Cannon and Sulzer follows:

A known volume of blood is delivered directly on to two or three times its weight of anhydrous sodium sulphate distributed over the bottom of the distilling vessels, a special test tube type of which is described and illustrated. This is placed in a water-bath at 40° to 50° C. and evacuated through a tube containing a known volume of standard potassium dichromate and an equal volume of strong sulphuric acid.

Distillation is allowed to proceed for fifteen to twenty-five minutes with the pump running, the vacuum then broken by opening the capillary inlet of the distilling flask and the absorption tube disconnected.

The contents are washed into a flask with sufficient water to dilute the sulphuric acid to less than 5 per cent, excess of 10 per cent potassium iodide solution added, and the liberated iodine titrated with starch and sodium thiosulphate.

This titration subtracted from the thiosulphate titer of the volume of potassium bichromate used gives the amount of the latter required to oxidize the alcohol and from the factor (1 c.c. N/1 potassium bichromate = 1.15 mg. alcohol) the amount of alcohol may be directly obtained.

Note.—The amount of anhydrous sodium sulphate should be such as to give a semi-liquid mass with the blood.

Piersol, Geo. M., Bockus, H. L., and Shay, H. H.: The Value of a Starch-Iodine Reaction as a Test of Pancreatic Function. Arch. Int. Med., March, 1926, xxxvii, 431.

The following modification of the Bassler method was employed:

A duodenal tube was passed into the duodenum on an empty stomach, preferably in the morning. When the tip of the tube was ascertained to be in the duodenum, 100 c.c. of 5 per cent Witte peptone solution was injected through the tube as an activator to the pancreas. The tube was clamped off for five minutes; then the duodenal contents were aspirated. The first fraction aspirated was used for the test, because Bassler states that this contains the largest portion of pancreatic juice and represents the sudden liberation of the stored up secretion of the pancreas.

The following reagents were employed:

Solution A, prepared as follows: In a beaker 2 gm. of cornstarch (Duryea) were placed; to this were added 100 c.c. of cold distilled water. This was mixed thoroughly and then heated. Under constant stirring the mixture was brought to boiling and then cooled.

Solution B, a 1 per cent sodium chlorid solution. The standard buffer solution mentioned in the original description of the test was omitted as it is now regarded as nonessential.

The standard Bassler reagent was then prepared by combining solutions A and B, as

follows: To Solution A, when cool, 25 c.c. of Solution B was added and enough distilled water to bring the volume of the mixture up to exactly 400 c.c. This standard reagent was freshly prepared each day.

In addition to these solutions, at first a N/25 iodine solution, kept in a bottle with a dropper cork, was prepared. After the first series of tests, this solution was changed to a twenty-fifth normal iodine solution, a strength which has been found more satisfactory.

The actual procedure employed in carrying out the complete test is a modification of the method devised by Smith in testing for ptyalin.

Ten tubes of equal size were placed in a rack and numbered from 10 to 1. Into each of the ten tubes there was accurately pipetted, with a 1 c.c. pipette graduated in hundredths, definite quantities of duodenal return, water and the standard reagent. The quantities in cubic centimeters of these materials that are added to each tube are shown in Table I.

TABLE I

QUANTITIES OF DUODENAL RETURN, WATER AND STANDARD REAGENT

	DUODENAL RETURN C.C.	WATER C.C.	BASSLER'S REAGENT C.C.	RESULT IN BASSLER UNITS PER 100 C.C.
Tube 10	0.1	0.9	4	20
Tube 9	0.11	0.89	4	18
Tube 8	0.125	0.875	4	16
Tube 7	0.14	0.86	4	14
Tube 6	0.17	0.83	4	12
Tube 5	0.2	0.80	4	10
Tube 4	0.25	0.75	4	8
Tube 3	0.33	0.67	4	6
Tube 2	0.5	0.5	4	2
Tube 1	1.0	0.0	4	2

When prepared and shaken, the rack was put in an incubator at 38° C. for forty minutes. The rack of tubes was then taken out; the contents of each tube were shaken and one drop of N/10 solution of iodine was added to each tube. Twenty minutes later the estimation of the amount of pancreatic amylase in terms of Bassler units was made according to readings obtained from the furthest tube to the left that was achromic. When there was no achromic tube, but instead one side of the rack was pink, and the other side green, the reading was taken between the adjacent pink and green tubes. The result in Bassler units was obtained by multiplying the number of the achromic tube by two. The normal reading is supposed to lie between 8 and 14 units, the unit being defined as the quantity of amylase that will digest 1 gm. of starch under the conditions outlined.

In the course of the investigation it was found that there are substances present in the test which are capable of absorbing such large amounts of iodine that it is unnecessary to have any starch digestant present in order to obtain achromic tubes. Hence, readings suggestive of the presence of amylase may be obtained even if there is no pancreatic juice at all in the material tested.

A method was devised, therefore, to determine the iodine absorptive power of the duodenal return after peptone stimulation which was responsible for the error.

A series of ten tubes numbered from 1 to 10 were taken. In each tube was placed 0.5 c.c. of duodenal return. To Tube 1 there was added 1 drop of N/25 iodine solution. The addition of iodine solution to each succeeding tube was increased by 1 drop; thus, 10 drops were added to Tube 10. The contents of each tube were mixed thoroughly and allowed to stand at room temperature for thirty minutes. This provided adequate time for saturation of the duodenal return with iodine. To each tube 4 c.c. of Bassler starch solution was then added and the tube with the largest amount of iodine which failed to show any starch iodine reaction was then determined. The number of this tube was multiplied by two. The result showed the number of drops of twenty-fifth normal iodine which 1 c.c. of that particular duodenal return was capable of absorbing. From this figure the iodine absorptive power of the quantity of duodenal return in each tube of the Bassler test was calculated; e.g., for Tube

10, one-tenth of the amount; for Tube 9, eleven-hundredths, and so on. The final step in the test, when the iodine was to be added, being reached, the calculated amount of iodine necessary to take care of the iodine absorption factor as previously determined plus one drop in excess of this amount was added to each tube. The determined amount was to allow for the absorption by the duodenal return, and the additional drop to react with any starch or products of starch digestion which still remained.

It soon became apparent that if the iodine was to be added accurately quantitatively, the drop method was not sufficiently exact. Therefore, the pipette was used for determining the iodine absorption factor. An accurately calibrated pipette was employed and for each drop used in the method outlined, 0.05 c.c. was substituted.

As thus modified the technic entire is thus summarized:

Modified Technic.—1. Pass duodenal tube. 2. Stimulate pancreas with 5 per cent Witte peptone solution. 3. Aspirate duodenal contents after five minutes. 4. Prepare 10 tubes as for test. 5. Incubate. 6. Determine iodine absorption factor: (a) Use 10 tubes with 0.5 c.c. of duodenal return in each tube. (b) Put 0.05 c.c. of N/25 iodine in Tube 1, increasing by 0.05 c.c. in each succeeding tube to 0.5 c.c. in Tube 10. (c) Shake; stand at room temperature thirty minutes. (d) Add 4 c.c. of Bassler starch solution to each tube. (e) Read achromic tube containing largest amount of iodine. (f) Calculate amount of iodine absorption for each tube in test. (g) Add calculated amount of N/25 iodine to each tube plus 0.05 c.c. in excess. 7. Read in twenty minutes.

This modified method gave consistent and uniform results with experimental animal material.

Jones, H. W.: Blood Transfusion in Pernicious Anemia. Jour. Am. Med. Assn., May 29, 1926, lxxxvi, 1673.

From a study of thirty cases the following conclusions are advanced: Life in those patients who present urgent symptoms can be saved. Unpleasant symptoms can be alleviated and the patient given added strength and hope. A so-called remission can be stimulated.

The patient can be carried along at a higher nutritional level with the hope that a remission will result. It is reasonable to assume that he has a greater opportunity to develop a remission when the blood is above 50 per cent than when it is between 20 and 50 per cent. What appears to be a cure in an occasional patient can be brought about. In order to accomplish these results the following points must be observed: 1. A small amount of blood (100 c.c.) should be given at the first transfusion to offset the possibility of death in a very few cases, unless the patient requires a larger amount to carry him past the danger point. 2. Whole blood should be used. 3. Moderate amounts of blood, from 350 to 450 c.c., should be given. 4. In the hemolytic stage, the amounts should be increased daily, beginning with 50 c.c. 5. Transfusion should be employed at four day intervals.

Wile, N. J., and Keim, H. L.: Effect of Treatment on Spinal Fluid in Cerebrospinal Syphilis. Jour. Am. Med. Assn., Oct. 24, 1925, lxxxv, 1269.

An analysis of 100 cases from which it is concluded: Deviations from the normal in the spinal fluid, in acute cases of cerebrospinal syphilis occurring during the exanthematous period, tend to be reduced to normal, parallel with clinical improvement following intensive treatment. This tendency toward normality in the abnormal spinal fluid is less noticeable in diffuse cerebrospinal syphilis occurring in the period of latency or with recurrences. In cases of parenchymatous disease of the cerebrospinal system, notably in tabes and paresis, there is little or no simultaneous tendency toward a reduction of the diseased fluid to the normal, even in cases in which clinical improvement is striking. The most easily influenced constituent of the diseased fluid in all types of cases is its lymphocytic content; less easily influenced, although still reducible in the acute cases, is the increase in globulin and albumin. The colloidal gold curve may be reduced to normal in the early cases, but is influenced with difficulty by treatment in the later phases of the disease. The least influenced by treatment

is the Wassermann reaction. This tends to be reduced to normal in the very early or acute cases, but seems to be uninfluenced and more likely to be fixed in the late cases than any other of the changed constituents of the spinal fluid.

Fisk, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus. Jour. Biol. Chem., December, 1925, lxvi, No. 2, 375.

Solutions needed:

N/10 Sulphuric Acid.—450 c.c. of concentrated sulphuric acid added to 1300 c.c. of water.

Molybdate I.—2.5 per cent ammonium molybdate in N/5 sulphuric acid. Dissolve 25 gm. of the salt in 200 c.c. of water. Rinse into a liter volumetric flask containing 500 c.c. of N/10 sulphuric acid. Dilute to the mark with water and mix.

Molybdate II.—2.5 per cent ammonium molybdate in N/3 sulphuric acid. Prepared as above, but with only 300 c.c. of N/10 sulphuric acid. (To be used only with blood filtrates in the determination of inorganic phosphate.)

Molybdate III.—2.5 per cent ammonium molybdate in water. As soon as any considerable amount of sediment (ammonium trimolybdate) has appeared in this solution, it should be discarded.

Ten per cent trichloracetic acid.—The quality of this reagent is of great importance. One brand tried contains some unknown impurity which retards the color development to a most pronounced degree. Merck's U. S. P. product is free from any such contamination, but contains a trace of phosphate. The amount of this must be determined in each sample or else the reagent purified by distillation.

Determination of impurity.—Arrange three tall beakers of 150 c.c. capacity on a piece of white paper. Into one of these (A) put 100 c.c. of water. In a second beaker (B) mix 85 c.c. of water, 10 c.c. of Molybdate I, and 4 c.c. of 0.25 per cent aminoaphtholsulphonic acid; the result should be a solution practically as colorless as water, without a trace of blue (otherwise one or more of the reagents already added contains phosphate). To the third beaker (C) add 40 c.c. of the trichloracetic acid solution, 45 c.c. of water, 10 c.c. of Molybdate II, and 4 c.c. of the sulphonic acid reagent, stirring thoroughly with a clean glass rod. Into B now run 1 c.c. of a dilute phosphate solution containing 0.005 mg. of phosphorus per c.c. and mix well. Proceed in the same way, adding 1 c.c. of this phosphate solution at intervals of not less than two minutes, until B and C appear to have the same color when examined from above. The volume of phosphate solution which must be added to bring this about, multiplied by 0.05, is the correction (in mg. per 100 c.c.) to be subtracted from the result in the analysis of blood.

Standard phosphate (5 c.c. = 0.4 MgP).—Dissolve 0.3509 gm. of pure monopotassium phosphate in water. Transfer quantitatively to a liter volumetric flask, add 10 c.c. of N/10 sulphuric acid, dilute to the mark, and mix. The standard keeps indefinitely.

Fifteen per cent sodium bisulphite.—The solution must be free from turbidity before it can be used. Freshly prepared sodium bisulphite solutions may not filter clear, in which case two or three days standing (before filtering) will be necessary. Keep well stoppered.

Twenty per cent sodium sulphite.—Use the crystalline sulphite ($Na_2SO_3.7H_2O$). Dissolve 200 gm. of this in 380 c.c. of water. Remove any suspended matter by filtration, and keep stoppered.

Aminonaphtholsulphonic acid, 0.25 per cent.—Dissolve 0.5 gm. of the dry powder (see next section) in 195 c.c. of 15 per cent sodium bisulphite, add 5 c.c. of 20 per cent sodium sulphite, stopper, and shake until dissolved. If the bisulphite solution is old, more than 5 c.c. of sulphite will be needed—in that event add more sulphite 1 c.c. at a time, shaking after each addition, until solution is complete. This reagent can be prepared in a few minutes (the powder need not be very accurately weighed), and if not left exposed to the air it should keep about two weeks. The solution is more stable the higher its acidity, hence no more sulphite should be added than is needed to dissolve the reducing agent.

1-2-4-aminonaphtholsulphonic acid.—This may be prepared from β-naphthol according to Folin's directions, with a single alteration. The final product, after washing with cold water, still contains some colored material. To remove this, the crystals on the filter, while still wet, should be further washed with alcohol as long as any color is extracted.

The reagent may also be obtained in satisfactory condition by one recrystallization of "technical" aminonaphtholsulphonic acid (Eastman Kodak Co.), as follows: Heat 1,000 c.c. of water to about 90° and dissolve in it 150 gm. of sodium bisulphite and 10 gm. of crystalline sodium sulphite. To this mixture add 15 gm. of the crude sulphonic acid, and shake until all but the amorphous impurity has dissolved. Filter the hot solution through a large paper (about 32 cm.), cool the filtrate thoroughly under the tap, and add to it 10 c.c. of concentrated hydrochloric acid. Filter with suction, wash with about 300 c.c. of water, and finally with alcohol until the washings are colorless.

The purified sulphonic acid should be dried in air with the least possible exposure to light, then powdered and transferred to a brown bottle.

Determination of inorganic phosphate in urine.—Measure into a 100 c.c. volumetric flask enough urine to contain between 0.2 and 0.8 mg. of inorganic phosphorus (usually 1 or 2 c.c.). Add water to bring the total volume to 70 c.c., followed by 10 c.c. of 2.5 per cent ammonium molybdate made up in N/5 sulphuric acid (Molybdate I), and 4 c.c. of 0.25 per cent aminonaphtholsulphonic acid. After the addition of each reagent the solution should be mixed by gentle shaking.

At the same time transfer to a similar flask 5 c.c. of the standard phosphate solution (containing 0.4 mg. of phosphorus), 65 c.c. of water, and the same reagents that were added to the urine sample. Dilute the contents of each flask to the mark, mix, and compare in the colorimeter after five minutes. With the standard set at 20 mm., 8 divided by the reading will give the inorganic phosphorus content of the sample in mg.

Determination of inorganic phosphates in blood.—Transfer to an Erlenmeyer flask four volumes of 10 per cent trichloracetic acid. While the flask is being gently rotated, run in 1 volume of blood, plasma, or serum—as the case may be—from a pipette calibrated for delivery (not contents). Close the mouth of the flask with a clean, dry rubber stopper, and shake vigorously a few times. Filter through an ashless paper.

Measure 5 c.c. of the filtrate into a tube graduated at 10 c.c. or a 10 c.c. volumetric flask. Add 1 c.c. of 2.5 per cent ammonium molybdate in N/3 sulphuric acid (Molybdate II), and finally (after mixing) 0.4 c.c. of the usual sulphonic acid reagent. Dilute to the mark and mix. The standard, to be prepared as nearly as possible at the same time, is identical with the standard used for urine (0.4 mg. of phosphorus in a volume of 100 c.c., or 0.2 mg. in a 50 c.c. flask with half as much of each reagent), so blood and urine may be read against the same solution. It should be noted that the molybdate reagent added to the standard is always the one containing N/5 sulphuric acid (Molybdate I), and is different from that used for the blood filtrate. The purpose of this is to compensate for the high concentration of trichloracetic acid in the filtrate.

The reading, as with urine, may be made in about five minutes, but it should be repeated a few minutes later if the color is particularly strong. To calculate the result in mg. of phosphorus per 100 c.c. of blood or other fluid (the standard being set at 20 mm.), divide 80 by the reading. From the figure so obtained subtract the correction for any phosphate which the trichloracetic acid may contain.

An inorganic phosphorus content of 2 mg. per cent is about the lower limit for convenient reading against the standard recommended, and a weaker color such as would be obtained by using half as strong a standard, cannot be read so accurately. Hence, as perhaps the least objectionable arrangement when the phosphate content of the blood is very low, the addition of a known amount of phosphate to the filtrate is suggested. This may be done, when a low result can be anticipated, before introducing the reagents. Otherwise, if the reagents are already mixed with the blood filtrate and the color is seen to be unusually weak, phosphate may be added then, before diluting to the mark—the less delay of course the better, but any time within five minutes will do, provided that twice as long a period is allowed before the final reading. A suitable amount of phosphorus to add is 0.016

mg., or 1 c.c. of a solution made by diluting 20 c.c. of the regular standard to 100 c.c. The calculation may then be made in the customary manner, and 1.6 mg. per cent subtracted from the result.

Method for total acid-soluble phosphorus in blood.—Five c.c. of the trichloracetic acid filtrate should be used if possible. Boil this down, over a microburner, in a large lipped Pyrex test tube (200 by 25 mm.) with 5 c.c. of N/5 sulphuric acid (or 2.5 c.c. of N/10) and a piece of quartz to prevent bumping. The bottom of the tube should be about 2 cm. above the burner tip. As soon as charring can be seen, or fumes appear, turn the flame down very low, so that the mixture barely boils, and heat until there is no further blackening. Now add 1 drop of nitric acid so that it runs down the wall of the test tube—it should not fall directly into the digestion mixture. If the color does not promptly disappear, add another drop of nitric acid in the same manner, and continue in this way until there is no color left. Nothing is gained by using a large amount of nitric acid. Ordinarily a single drop will be enough, and then about thirty seconds further boiling with the same low flame (to remove most of the nitric and nitrous acids left) will complete the ashing process.

Cool the tube under the tap, rinse the contents into a 50 c.c. volumetric flask with 35 c.c. of water, add 5 c.c. of Molybdate III, (2.5 per cent ammonium molybdate in water alone), and 2 c.c. of the reducing agent. Dilute to the mark, and proceed as usual, reading against the standard that has been described before. Four hundred divided by the reading will give the desired result in mg. per 100 c.c. of blood.

The analysis may be made with 1 c.c. of filtrate, using 1 c.c. of N/5 sulphuric acid. The procedure is then otherwise the same as that described above, except that the final dilution must be 10 c.c. (instead of 50) and the reagents diminished in proportion. It is safer, in this case, to use a smaller test tube (about 10 mm. in diameter) for the digestion. The most probable cause of loss of phosphate is superheating at the edge of the meniscus, which should consequently be as far removed as possible from the source of heat.

Tomb, J. W., and Maitra, G. C.: A New Method of Isolating and Cultivating Vibrios from Feces, Especially Suited for the Detection of Vibrio Carriers in Field Work. Indian Med. Gazette, February, 1926, 56.

In ordinary enamel bowls are placed 250 c.c. of sterilized tank water, 1 per cent of common salt, 1 c.c. of 1 per cent sterile peptone being added just before the inoculation with feces.

Specimens are collected by inoculating 1 by 6 inch test tubes conntaining 10 to 15 c.c. of 1 per cent salt solution. This solution tends to inhibit the usual intestinal flora while vibrios multiply temporarily.

After arrival in the laboratory, or after six to eight hours at room temperature, about six large loopfuls are inoculated into the enameled bowls and daily transplants made into peptone, bile-salt agar, etc. If no vibrios are found within a week the bowls are discarded.

Badoux, V. E.: The Benzochol Precipitation Reaction in the Serodiagnosis of Syphilis. Annal. de Malade Ven., January, 1926, xxi, 21.

"Benzochol" is the name given to the following mixture:

1. A cholesterinized extract of bull heart (the ordinary Sachs-Georgi antigen).

2. A 10 per cent alcoholic solution of benzoin resin prepared by shaking the resin for twenty-four hours and filtering off the residue.

Benzochol consists of 10 c.c. of extract and 3 c.c. of resin solution "diluted to one-twentieth of this concentration with 0.85 per cent saline."

It is prepared by rapidly adding 9.5 c.c. of normal saline to 0.5 c.c. of benzochol, making a milky suspension.

The Test.—Place 0.1 c.c. of inactivated serum in a 10 cm. by 15 mm. tube and add at once 0.5 c.c. of the benzochol test solution. The tubes are then shaken for ten minutes in a shaking machine and a reading made. A second, final reading is made after incubation at 38° C. for thirty to forty-five minutes.

The reaction is similar to the Sachs-Georgi and gives comparable results but its practical value is impaired by the instability of the benzoin resin solution which may rapidly lose its antigenic value.

Felton, L. D., and Bailey, G. H.: The Specific Precipitates Obtained from Antipneumococcus Serum and Antibody Solution by the Soluble Specific Substances of Pneumococci. Jour. Infect. Dis., February, 1926, xxxviii, No. 2, 145.

With appropriate amounts of soluble specific substance all the protective substance in Type II antipneumococcus serum may be precipitated.

The precipitate is insoluble in water, sparingly soluble in 3 per cent sodium chloride, and very soluble in dilute acid and alkali when the salt substance is low. Sodium carbonate in concentration of M/100 is the best dissociation agent but the resulting solutions have no demonstrable protective power.

Felton, L. D., and Bailey, G. H.: Biologic Significance of the Soluble Specific Subtances of Pneumococci. Jour. Infect. Dis., February, 1926, xxxviii, No. 2, 131.

Experiments are recorded which show that the protective substance of antipneumococcus serum and antibody solutions are neutralized both in vitro and in vivo, the neutralization being approximately in proportion to the amount of soluble specific substance present.

Felty, A. R., and Heatley, C. A.: The Nasal Passages in Lobar Pneumonia, a Clinical and Bacteriologic Study. Jour. Am. Med. Assn., April 17, 1926, lxxxvi, 1195.

In a series of sixteen cases of lobar pneumonia, pneumococci of corresponding type to those found in the sputum were isolated from the middle fossae of the nose in every case.

The nasal passages in fifteen of the sixteen cases were examined with the nasopharyngoscope; of these, all showed hyperemia of the mucous membranes, and six had signs of acute suppurative sinusitis. In eight cases, anteroposterior roentgenograms of the sinuses were made; in seven cases, clouding of one or more of the sinuses was observed.

In this small series of cases, acute pneumococcal sinusitis was a frequent accompaniment of lobar pneumonia.

Anderson, W. T.: Investigations Concerning Content of Calcium in Serum. Hospitalstid, Dec. 31, 1925, lxviii, 1177.

The author conducted a series of researches which involved determination of the content of calcium in serum in accordance with the method devised by Waard. In these it was found that normal values ranged between 10 and 11.5 mm. per cent, and appeared extraordinarily constant in each single individual. The amount of calcium in serum was modified by means of employment of light baths.

In bronchial asthma low values were encountered during paroxysms. No actual reduction in content of calcium in serum was noted in Basedow's disease. Effects of treatment with roentgen rays were suspected in instances in which a decrease occurred. A variable diminution was remarked in renal insufficiency, while in arterial hypertension without renal symptoms the quantity of calcium contained in the serum proved normal or was slightly increased.

Ishii, O.: Studies in the Wassermann Test for Syphilis. Am. Jour. Syph., January, 1926, x, 1.

A report of studies conducted with reference to technical principles and the influence of natural antisheep amboceptor in human serum which, in general, corroborates the findings of other workers.

The following method was devised for the absorption of natural hemolysin:

The sheep corpuscles are washed thoroughly three or four times by centrifuging with about five volumes of saline solution. The concentrated cells are then dehydrated by adding

five times the volume of absolute or 95 per cent alcohol and thoroughly mixed. The supernatant fluid is discarded after ten minutes and the cells are allowed to dry on a glass plate in the incubator at 37° C., or at room temperature, for about ten minutes. They are then ground into a fine powder by the mortar and pestle.

In the test, one volume of dried sheep corpuscles is mixed with about four or five volumes of active or inactivated human serum, shaken well and allowed to stand for a few minutes at room temperature. This mixture is then centrifugalized for five minutes at moderate speed and the serum drawn off. This procedure absorbs the natural antisheep amboceptor together with the hemoagglutinin and has no effect on the complement.

Bernheim, A. R.: The Significance of Variations of Bilirubinemia. Arch. Path. and Lab. Med., May, 1926, i, No. 5, 748.

A study of 485 unselected cases using a modified Meulengracht test.

Observations of the variations in bilirubinemia are chiefly directed to the study of liver function, but bilirubin, besides being a product of the liver, is a normal constituent of the blood, and as such plays a rôle of some importance in a number of conditions not primarily concerned with the liver.

The accuracy of the test for the determination of the icterus index and the facility with which it is performed make it available not only for investigations in hospitals, but also for the use of the outside practitioner.

As a diagnostic and prognostic aid in a number of diseases, the determination of the icterus index is a procedure which may be said to hold equal rank with other laboratory tests, such as those for blood sugar, urea nitrogen and creatinine, with an advantage over these tests in that it is simpler to perform.

It is seen that under controlled conditions in which the liver is stimulated, blood sugar and bilirubin values bear a definite relation to each other. Explanations of these findings are presented with due recognition of the conjectural element involved, and with the hope that further evidence may either substantiate them or show why they are incorrect.

Hypobilirubinemia (below 2.3) may be noted where there is a reduction of red cells.

In diabetes the icterus index is high (average 10).

Doan, C. A.: Recognition of a Biologic Differentiation in the White Blood Cells. Jour. Am. Med. Assn., May 22, 1926, lxxxvi, 1593.

A study of white blood cells by a supravital technic in an attempt to find an explanation for the reactions noted in rabbits transfused with matched blood.

Technic.—First, it is essential that the slides and cover glasses for the supravital preparations be free of grease and neutral in reaction (nonacid). Glassware is placed in a saturated solution of potassium bichromate in concentrated sulphuric acid for three or four days, and then washed in running tap water for twelve hours. It is rinsed in three changes of distilled water; the slides stand overnight in the last change. It is stored in 80 per cent alcohol. It is dried with clean, new gauze and flamed. If a dye is used (it is not essential), vital neutral red is recommended. Grubler's prewar, vital neutral red is made up in saturated solution in neutral absolute alcohol. Twenty-five drops of the saturated solution in 5 c.c. of absolute alcohol gives a nontoxic concentration of the dye for use in examinations of normal blood. The slides are flooded with the dye and then drained; they are allowed to dry in the upright position, which insures a thin even film of the dye on the slide. A film of blood made on such a slide, when kept in the warm box at 37.5° C., will show viable cells for as long a period as a film made without the dye. Moreover, the dye makes the individual cells much more easily visible and the identification of cell types more certain. The prime requisites, it is to be remembered, however, are motility of the cells and freedom from any technical cellular depressant. The weight of the cover slip will cause the drop of blood to spread to the desired extent, provided the glassware is clean. It is sealed with petrolatum of a higher melting point than the temperature at which the examination is to be made. Either a warm box or a warm stage, preferably the former, may be used. The uniformly constant temperature conditions are an essential.

The cover slips are prepared in a similar way. They are flooded with the citrated or oxalated plasma or blood serum that has been secured for grouping the red blood cells, and drained to one corner in the vertical position, the drop of plasma that forms there being carefully removed with a fine capillary pipette. This is necessary in order that the unevenness attendant on the drying of a large drop of plasma at that point may be avoided. From the finger or ear of the person whose white blood cells are to be tested, a drop of blood is taken directly on the cover slip, prepared with the dried serum, and dropped gently on the slide. If the blood does not promptly spread into a thin, even film, slight manipulation from the side, never a pressure from above, may be used. Immediately, on the same slide, and from the same or a subsequent drop of blood, the control film is made with a plain cover slip. Both are sealed with petrolatum, and, under proper temperature conditions, the control slip is looked at first, to make certain of the viability of the leucocytes. It is the comparison or contrast between the number of viable cells in the two preparations on which depends the determination of the compatibility of the plasma in question. Oxalate and citrate anticoagulants in themselves do not affect the white blood cells in this test. However, as will be seen when a blood is leucotoxic, citrated or oxalated plasma is doubly destructive to the susceptible white cells. At certain times there are physiologic showers of the "nonmotile" leucocytes, described by Sabin and her coworkers in 1925, which must be differentiated from the white cells specifically affected by the incompatible blood plasma. Both by the difference in physical characteristics and by their presence and number in the control film, it is readily possible to eliminate such preparations as pseudopositives. If the control film, at any time, contains a high percentage of the "nonmotile" leucocytes, it is advisable to make a second duplicate preparation, as this particular phase of the polymorphonuclear leucocyte is transitory and does not reflect the plasma effect so readily as does the actively motile form.

It was noted that certain serums have a more disintegrative effect than others on the white cells.

There seems to be a definite incompatibility between the blood plasma of certain individuals and the white blood cells of others, as shown by simple in vitro tests.

A definite biologic classification of individuals according to white cell compatibility is difficult, because of the fact that at least twenty-seven different combinations are possible, many of them not infrequent. Approximately 40 per cent of the group of forty persons investigated may be considered universal donors (group A); 5 per cent seem to have cells susceptible to all, or nearly all, plasma (group Z); nine different combinations are represented in the remaining 55 per cent, to which no arbitrary group designation has been assigned at this time.

The added precaution of ascertaining the relative compatibility of plasma and white cells by direct matching, preliminary to transfusion, particularly in cases in which extreme weakness makes imperative a successful operation, is advisable.

Lash, **A. F., and Kaplan, B.: Puerperal Fever, Streptococcus Hemolyticus Toxin and Antitoxin.** Jour. Am. Med. Assn., April 17, 1926, lxxxvi, 1197.

Experiments with hemolytic streptococci isolated from the blood in puerperal fever. The toxic substance in the Berkefeld V filtrate of such cultures is a toxin because:

1. There is a latent period between injection and reaction.
2. It is heat labile.
3. It is neutralized by antitoxin and not by non-immune serum.
4. Precipitin tests show the development of immune bodies by the toxin.
5. Repeated injections of the toxin give rise to the production of a specific antitoxin.

Nevin, **M., Bittman, F. E., and Hazen, E. L.: Unsuccessful Attempts to Cure or Prevent Tuberculosis in Guinea Pigs with Dreyer's Defatted Antigen.** Am. Rev. Tub., February, 1926, xiii, 114.

The defatted antigen described by Dreyer produced no beneficial results, either prophylactic or therapeutic, in guinea pigs. On the contrary, animals treated with the vaccine died earlier than the untreated controls.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Radium: Its Therapeutic Uses in General Practice**

THE author voices the regret so often expressed by radiologists who are dependent in great part for their work upon material referred from surgeons, that were radiotherapy given an untrammelled opportunity without antecedent surgical intervention, the results would be even better. This applies particularly to malignancy. Surgeons have not as yet reached the stage where they are willing to turn the case over to the radiologist for entire charge. If the case is still considered operable, even faintly so, the only chance that the x-ray or radium therapist has is along the line of preoperative and postoperative care. The time will come when he will have better opportunity to demonstrate the real values of his method of treatment.

The author includes sections devoted to the properties of radium, a brief historical recapitulation, description of induced radioactivity, discussion of technic and dosage. The major portion of the book is devoted to therapeutic methods and results in specified conditions. Radium has its place in the treatment not only of malignancy but in certain blood diseases such as leucemia and in the treatment of naevi, moles, papillomata, adenomata, warts, certain diseases of the uterus, goiter, actinomycosis, fibroids, acromegaly and other conditions. He finds that radium has more effect on metastases than on local recurrences or secondarily infected lymph glands. Malignant disease appears to be more susceptible to radium therapy when the lesion is "backed up" by bone. On *the other hand,* when the disease is in the bone itself the results are not as good. In prophylaxis he usually gives radium exposure two or three days prior to operation. A second course is given from four to six weeks following operation. Following breast operations he recommends the routine implantation of radium tubes in the region of the supraclavicular gland for a period of twenty-four hours.

Squamous epithelioma of the tongue, esophagus and larynx is not very satisfactorily treated with radium, but the pain is usually relieved in these conditions. Epithelioma of the lower lip responds very readily. The author's results in carcinoma of the rectum have not been so satisfactory as in some other conditions, but they probably average up with other methods. Carcinoma of the cervix uteri responds splendidly. If taken early enough, the results appear better than by any other method and one is enabled to do away with the 20 per cent immediate operative mortality. The results with rodent ulcer are distinctly superior to those obtained by surgery.

It is surprising to read that the author does not report particularly good results in fibroid tumor of the uterus. This presumably is due to difficulty in access, for deep x-ray therapy gives excellent results.

There is a close parallelism between the results obtained by radium treatment and those obtained by a skilled x-ray therapist. With the somewhat greater flexibility in x-ray technic and with the advent of deep therapy, the x-ray will usurp much of the work that has been done by radium.

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*Radium: Its Therapeutic Uses in General Practice. By G. H. Varley, M. D. (Oxon.). Late Clinical Assistant to the Radiological Department St. George's Hospital. Cloth. Pp. 103. Oxford Univ. Press, 1924.

*International Clinics, March, 1926**

THIS volume follows the time-honored custom of *International Clinics* of presenting articles by recognized authorities from this country and abroad on subjects of diversified interest.

Dr. Reisenstein of Syracuse contributes a very good didactic discussion of the diagnosis and treatment of cardiac arrhythmias. It is well illustrated with electrocardiographic tracings. G. Paul La Roque, of Richmond, presents his views of the treatment of appendicitis in no uncertain terms. We are inclined to think of the surgical treatment of appendicitis as so thoroughly established as to permit of no further discussion. However, Dr. La Roque raises several pertinent questions, some of which will be found perhaps slightly embarrassing to the surgical enthusiasts who insist that with the establishment of the diagnosis, operation must of necessity follow immediately irrespective of the various local factors that should call for individualization in treatment. At the same time several of the points raised should be disconcerting to the physician who precedes the surgeon and who too often when called upon to treat a patient with abdominal pain will prescribe a cathartic, a soft diet or even an enema. The article by Dr. La Roque alone should make the volume worth while.

We learn from Professor Plut, of Munich, that general paresis is being treated with relapsing fever as well as with malaria. The former has the advantage that the superimposed infection does not get out of control and that the virus for inoculation may be carried as a stock preparation in laboratory animals. It has this disadvantage, that the patient having once recovered from relapsing fever cannot be reinoculated. The results with this treatment appear to compare very favorably with those following malaria.

In this as in other volumes of the series, a section is devoted to electrotherapy and physiotherapy.

In the first volume for each year *International Clinics* incorporates a section on the progress of medicine during the preceding year. Under this heading Doctors Cattell and Coupal bring out that while no outstanding or spectacular single advance was made during 1925, the practical and clinical application of laboratory discoveries of the second order has distinctly improved our methods of treatment of certain common diseases such as gall bladder disease, paralytic postoperative obstruction, postoperative hypoglycemia and hypotension. They also point out that notwithstanding the valuable diagnostic procedures which the laboratory has given to medicine, the trend has been back to careful physical diagnosis and the treatment of the individual rather than the disease from which he is suffering. The reaction against too much dependence upon laboratory methods alone, continues. There has perhaps not been any diminution in the use of special laboratory tests, but a more intelligent interpretation of their advantages and limitations and a greater consideration of the cost of time and money as compared to their actual values.

The subject of recent progress in surgery is covered by Balfour and Reid.

Prescription Notes†

A SMALL volume scarcely larger than a prescription pad, bound in black leather which may be easily carried in the coat pocket. Most of the leaves are blank pages for the insertion of favorite prescriptions, new prescriptions that may be picked up from time to time, and notations concerning treatment. As an undergraduate student the reviewer had a little pocket notebook containing special prescriptions to which he added particularly during his intern year. Even today he refers to it for some long forgotten combination.

It must have been with the same idea in mind that Dr. Tatum made up the small volume under consideration. In the first 22 pages we find handy information such as instructions for the proper construction and use of vehicles, general rules regarding incompatibilities, rules of dosages for children, weights, measures, a Latin Lexicon, and a list of the more important preparations described in the U. S. Pharmocopeia with their dosages.

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*International Clinics. A Quarterly of Illustrated Clinical Lectures and Especially Prepared Original Articles. By Leading Members of the Medical Profession Throughout the World. Cloth. Pp. 309. J. B. Lippincott Co., March, 1926, Philadelphia.

†Prescription Notes. By Arthur Tatum, Ph.D., M.D., University of Chicago. Cloth. Univ. of Chicago Press, 1925.

*The Clinical Interpretation of the Wassermann Reaction**

THE author requires no introduction to our readers. The reviewer would characterize this book so far as the complement-fixation test for syphilis is concerned as the clinician's *vade mecum*. Only enough of the actual technic of the reaction is described so that the reader will have a clear understanding of the procedures applied. In every sense the work lives up to its title.

The author points out all possible sources of error, both in the hands of the serologist and those of the clinician and suggests the appropriate measures for avoiding these errors. The significance of anticomplementary reactions and of the provocative reaction are discussed. The Wassermann reaction in pregnancy and in the newborn is elucidated.

A chapter is devoted to the complement-fixation reaction in diseases other than syphilis. The quantitative Wassermann reaction such as that elaborated by Kolmer is of particular value in treatment, since it gives a much more clearly defined picture of the progress of recovery.

We would recommend particularly to all physicians the reading of the chapter on the clinical interpretation of the cholesterin-plus reaction, and that on the provocative reaction. It is our opinion that the latter is applied inaccurately by the clinician in from 60 per cent to 80 per cent of cases.

After giving a general discussion of the complement-fixation reaction, the author describes those methods which are acceptable and reliable, and he mentions the more recent precipitation reactions. Concerning the latter he emphasizes particularly their shortcomings.

The last chapter deals with the proper methods of collection of specimens for the complement-fixation test.

The great value of the book will be as a desk reference manual to use when in the individual case a question arises as to the significance or interpretation of a complement-fixation reaction.

The Private Practitioner as Pioneer in Preventive Medicine†

SIR GEORGE NEWMAN in his Hunterian oration for 1926 reviews the contributions to the prevention of disease made by those practitioners of medicine who lived in England in the Hunterian period or more broadly in the eighteenth century. Still early in the renaissance of modern medicine, these men were not alone interested in the cause of disease and in its cure, but even then they were taking active steps toward its prevention. Their interest was in epidemic disease such as plague, influenza, typhus, smallpox and in such conditions as puerperal sepsis and chronic alcoholism. During the eighteenth century the more thoughtful and investigative of the British physicians introduced the principles of medical notification, of isolation, of fumigation and disinfection. They advocated an improved and enlarged dietary with restriction of alcoholic consumption.

They began the reformation of midwifery and first systematically attacked the problem of infant mortality. They lent their support to the establishment of dispensaries, hospitals, and medical schools. They laid the early foundations of immunology.

To us today the most outstanding feature of the contributions of the eighteenth century to preventive medicine in England was the discovery by Jenner of vaccination. The description which Dr. Newman gives of the results of alcohol consumption in the early part of the century is most interesting in view of present conditions in the United States. "Drunkenness abounded. By 1720 we had become a nation of tipplers. The nobility patronized brandy, the well-to-do middle classes drank a newly introduced spirit called rum; the working classes imbibed a characteristic spirit known as British gin. The consumption of gin was enormous. When certain restrictions which had been temporarily imposed were repealed in 1733, England touched a lower depth of inebriety than ever known before or seen since. The prevailing intemperance was the most momentous event of the eighteenth century.

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*The Clinical Interpretation of the Wassermann Reaction. By Robert A. Kilduffe, A.B., A.M., M.D. Cloth. Price $2.50. Illustrated. Pp. 203. Lea & Febiger, Philadelphia, 1926.

†The Private Practitioner as Pioneer in Preventive Medicine. Being the annual oration of the Hunterian Society. 1926. By Sir George Newman, K.C.B., M.D., D.C.L. Paper. Pp. 47. Oxford Univ. Press.

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EDITORIALS

The Epidemiology of Pneumonia

IN SPITE of the vast amount of consideration and study which have been expended upon it, pneumonia still maintains the rank conferred upon it by Osler as "Captain of the Men of Death."

Lacking effective means for its specific treatment, and in common with the general trend of modern medicine, it would seem that the direction of attack must be, for the present, at least, oblique rather than frontal and directed toward the evolution of prophylaxis—if this be attainable.

Studies concerned with the spread of this justly dreaded infection are, therefore, of great interest and such an analysis has recently been published by Rosenau, Felton and Atwater[1] which it is the purpose of this editorial to summarize.

Their study comprises 28 cases, carefully typed, and the records of 450 persons examined in an effort to define the sources and modes of infection with the pneumococcus.

[1]Rosenau, M. J., Felton, L. D., and Atwater R. M.: An Epidemiologic Study of Pneumonia and Its Mode of Spread, Am. Jour. Hygiene, May, 1926, vi, No. 3, p. 463.

As pointed out by the authors, while man is the source and reservoir of the pneumococcus, the fact that pneumonia is a group of specific diseases complicates the study of its epidemiology as does the rôle of predisposing causes and accessory factors influencing its occurrence and virulence, all of which must be investigated before epidemiologic studies can be considered complete.

In a study of the sources of infection the distribution of pneumococci in 180 normal individuals in Boston was investigated during the winter of 1923-24, these representing 26 families in whom there was no pneumonia.

The results of this study are tabulated below.

AGE	TYPE I	TYPE II	TYPE III	TYPE IV	NO PNEUMOCOCCI FOUND	PERCENTAGE OF CARRIERS
0– 9	1	0	2	26	27	52
10–19	0	3	4	24	35	47
20–29	0	0	2	9	10	52
30–39	1	0	0	8	14	39
40–49	0	1	1	6	7	53
50 and over	0	0	0	3	2	60
Percentage of each type	1%	2%	5%	41%	51%	49%

In a similar study of 270 normal individuals in contact with 28 cases of lobar pneumonia the results are shown below. It is to be noted that all of the 28 cases of pneumonia were either Type I or Type III. Therefore, the occurrence of Type II in the exposed group would be expected to correspond with the occurrence of Type II in the nonexposed group—and this is the case.

AGE OF CARRIER	TYPE I	TYPE II	TYPE III	TYPE IV	NO PNEUMOCOCCI FOUND	PERCENTAGE OF CARRIERS
0– 9	2	0	2	17	47	41
10–19	2	1	3	15	14	60
20–29	2	0	5	14	37	36
30–39	4	2	3	14	22	51
40–49	0	1	3	11	19	44
50 and over	0	2	6	15	14	62
Percentage of each type	3.6%	2.2%	7.9%	31%	55%	45%

It is regarded as significant that the proportion of Types I and III is distinctly greater among those exposed to these cases.

These studies show that there is no relation between the age of the contact and the distribution of the pneumococci. The degree of intimacy in the contact was of perceptible influence as might be expected.

Virulence studies of pneumococci isolated from cases and carriers furnished some evidence that the former were rather consistently more virulent which is further borne out by evidence tending to show that contact with cases of pneumonia is more productive of positive contacts than is contact with healthy carriers.

The inherent difficulties of the problem are shown by the fact that of fourteen healthy carriers in the control group not one gave a history revealing the source of the fixed type organism present.

The study tends to indicate that cases of Type I and III pneumonia are more apt to produce carriers than carriers are apt to produce these types although carriers may produce homologous types of the disease in small numbers.

As far as the evidence goes the disease seems mainly spread by contact.

—*R. A. K.*

Details

EVEN a casual glance at the Current Index of Medical Literature suffices to indicate the relatively enormous amount of information of varied kind—and value—which accumulates in the course of a single year and with which the alert physician, anxious to keep abreast of the newer developments, must have, at least, a speaking acquaintance.

It is manifestly impossible for any one individual to familiarize himself with the innumerable minutiae concerned with medical progress and, indeed, it will be highly commendable if, by effort and application, he is enabled to keep in touch with the salient advances in the field in which his particular skill or interest lies. The important thing is to know where to find the information sought and how to apply it when found.

With this end in view, one is at times tempted to envy the verbal fecundity which produced the total ruin of the fabled jackdaw of Rheims when, having sought and found a particular paper one finds that at least three or four readings coupled with much cogitation are necessary to excavate the particular point at issue.

Certainly a writer describing a new method does so with the intention of making it available for the use of others; another, presenting the conclusions formulated from his own experience or investigations, believes them worthy of consideration.

Why not, then, present them in such fashion that others may repeat or utilize the work, or properly evaluate the conclusions drawn?

Perhaps this fault of lack of detail and clarity in the description of procedures is most common in the description of methods.

It is unprofitable—and annoying—to read that so-and-so's method was employed when, perhaps, the method has undergone several modifications and nothing is said as to which was employed; or to say that such and such a technic was used when, as a matter of fact, some modification of it was really used.

It is somewhat difficult to attempt to try a new method, for example, and to be confronted at the outset with loosely written reagent formulae.

Attention has been called to this especially in connection with staining methods[1] in the description of which the following type of formula is frequently seen:

[1]Stain Technology, April, 1926, i, No. 2, p. 49.

"Alc. sol. fuchsin 1 part
Water 10 parts"

What does this formula mean? What strength fuchsin? What strength alcohol? What fuchsin? Are the proportions measured by volume or weight?

How much more satisfactory to write:

"Sat. alc. sol. basic fuchsin (dye content 88%) 1 c.c.
Distilled water 10 c.c."

Still more ambiguous and unsatisfactory are statements such as: "Boil with dilute sulphuric acid." "Add a few drops of water" and so on.

Examples in both clinical and technical papers could be cited almost ad infinitum.

If it is worth saying at all it is worth saying well—and clearly!

—*R. A. K.*

Errata

Article by Caven and Cantarow, October, 1926, page 76.

Page 76, title should read: A Method for the Determination of Calcium in Whole Blood.

Third line, first paragraph, word *devised* should be *used*.

The following paragraph should be substituted for the last paragraph on page 76 and the first paragraph on page 77.

Following hemolysis and centrifugation a small precipitate is thrown down with the precipitate of calcium oxalate, which we believe to consist of the stroma of the disrupted red cells. Varying amounts of added calcium have been successfully recovered. Using this method values ranging from 6.5 to 9.5 mg. per 100 c.c. of whole blood have been obtained. These figures are no doubt higher than the actual calcium content, probably due to the oxidation by the permanganate of some of the constituents of the stroma. This method was used to obtain a curve of changes in blood calcium following the injection of parathyroid extract, rather than to determine the absolute calcium content. It was found satisfactory for this purpose since any error caused by the constituents of the stroma should be practically constant.

Page 77, first reference, second line, the journal reference after the word *Report* should read, Jour. Am. Med. Assn., 1926, lxxxvi, 1683; second reference, third line, journal reference should read, Arch. Int. Med., 1926, xxxviii, 502; third reference, fourth line, add p. 461 to the reference.

In the article "Studies in Toxicologic Chemistry I. The Detection of the Opium Alkaloids by Selenious-Sulphuric Acid: The Specificity of the Reagent for the Phenolic Group" by Victor E. Levine, June, 1926, issue, the fourteenth line from the top of page 811 should read: Levine, 0.01 milligram of morphine equivalent to 0.025 milligram of morphine sulphate.

The twelfth phenol in Table II, page 812, should have the following item:

Diathesin (o-hydroxybenzyl alcohol) ---------- Purplish to cherry red in sulphuric acid alone; dark brown in selenious-sulphuric acid.